

The French: are they legal?

It is ironic that at a time when Professor Jacques Monod has to go to extraordinary lengths to keep the Institut Pasteur alive, the French government should be spending several hundred million pounds annually on nuclear defence. The continuance of a vigorous and controversial French programme of atmospheric weapons testing is bound to stimulate more argument this summer both within France and beyond her borders and so the appearance of a summary by the Stockholm International Peace Research Institute (SIPRI) of the legal questions of French activities is a welcome event (*French Nuclear Tests in the Atmosphere: The Question of Legality*, SIPRI, Stockholm, March 1974).

France started her military nuclear programme in the 1950s and by 1960 was able to test fission devices in Algeria, at first in the atmosphere but from 1961 underground. In 1966 testing was moved to the South Pacific and has continued at the rate of several devices a year in the atmosphere since then.

Concerted attempts to stop atmospheric testing go back twenty years and it is undeniable that public opinion was a major factor in securing the signing of the Partial Test Ban Treaty in 1963 by all nuclear and potentially nuclear powers except France and China. Since then signatories have been restricted to underground testing. Protests against the French activities were relatively insignificant until about a year ago, largely no doubt because the French test site is remote from large centres of population.

It was last summer's series, announced in advance, which drew the sharp criticism, at both national and personal levels. As it turned out that the devices were modest but in expectation of megaton tests several countries raised objections, of which the most interesting and substantial were those of Australia and New Zealand who proceeded against France in the International Court of Justice.

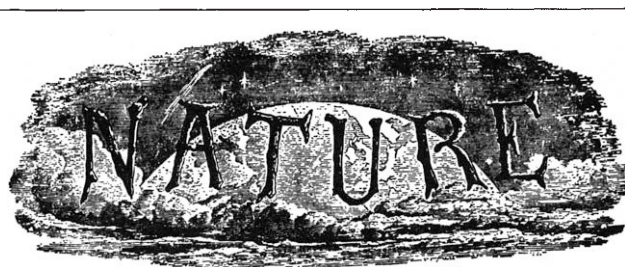
The International Court was told that tests violated, among other things, the rights of Australian and New Zealand people to be free from radioactive fall-out and to have free passage on the high seas. It was asked to order the French to desist from testing. The French replied that the court was not competent since the matter was one of national defence and France had already declared such matters to be beyond the court's jurisdiction. The French government was not represented at the hearings and when the court concluded, by a vote of eight to six, that "the French government should avoid nuclear tests causing the deposit of radioactive fall out" France chose to ignore the order. The French government prepared a White Paper justifying its stance legally and scientifically in strong terms.

The danger with questions of national policy is that they are not easily broken down into neat compartments. Few actions can be at the same time politically acceptable, legal, moral and safe. Furthermore, how safe is safe? If the French action is dealt with on grounds of legality and harm to the environment, the outcome will be muddy. As it was, the court decision was by a vote of eight to six and French scientists could be found to match Australian scientists on the safety issue. Those who seek a clear resolution of this conflict by law or scientific discussion are pushing in the wrong direction. Nor is the French government on any firmer ground in saying that international hostility comes from a political desire to impede French defence policy and spite French independence. Many of the most vocal countries have little concern for French defence policy and many of those who do worry about it registered no protest. The issue at stake is not whether the possession of nuclear weapons is justified or sensible. It is, in the final analysis, whether firing nuclear explosions in the atmosphere is a civilised or moral thing to do in 1974.

It is very difficult to argue such an issue through reasoned debate. Nevertheless, it is possible for a national consensus to be reached and it is incumbent on governments which sense such a feeling to act appropriately. Labour in opposition expressed disapproval.

As for the French, the signs are that things are changing under increasing moral pressures. There are ample rumours that an underground test site is being developed at Mururoa atoll, maybe to be ready by 1975.

100 years ago



Note on apparently useless Colouring in the Flowers of a Fumitory (Fumaria capreolata var. pallidiflora, F. pallidiflora Jord.)

I observe that in this plant at Mentone the flowers attain their brightest colouring after the ovaries are set, and when fertilisation is no longer necessary, or indeed possible. During the period previous to impregnation, the flowers are pale and nearly white, and the pedicels erect or horizontal; afterwards they become pink, or even crimson, and the pedicels are recurved, and the colour of the petals, which retain their form and position until the ovary has nearly attained its full size, intensifies with the lapse of time.

If the reverse had been the case there is little doubt that we should have regarded the bright colouring as specially adapted to attract insects, and as existing for that purpose, insects being, according to Prof. F. Hildebrand,* important agents in the fertilisation of fumitories; but here, as the brighter flowers are those which no longer need or are capable of profiting by the interference of insects, this explanation ceases to be possible.

This little fact, therefore, would seem to be one which might be classed with those which teach us that, side by side with the developments and modifications which are plainly beneficial to the organism of which they form a part, there are others, which, as far as we can see, are neither useful nor harmful to their possessor, though they may, and frequently do, supply features which especially attract our attention and admiration.

J. TRAHERNE MOGGRIDGE



Cancer and leprosy in South Asia

Professor E. J. Ambrose, of the Chester Beatty Research Institute, London, describes in particular the work being carried out on leprosy and cancer in Bombay.

MOST of the major health problems in South Asia are due to infectious diseases into which research is highly advanced, and the control of the diseases has mainly become a public health problem. There are two exceptions to this—cancer and leprosy. Cancer is a disease which, with the increase in life expectancy, affects a progressively increasing proportion of the population. It is already the fourth ranking killer among the over-45s in Bombay. Cancer research is now an intensive worldwide activity but there are many unsolved problems; as they occur in South Asia these have a different emphasis from those of western countries, for example the disease may be very advanced before the patient reaches a specialist centre requiring more radical forms of treatment and therapy, and the patient may be more sensitive to drugs (because of dietary factors) than is usually the case in the West; also, cancers at certain sites (oral and pharyngeal cancer from chewing habits, for example) are more prevalent.

Tata Memorial Hospital

The Tata Memorial Hospital in Bombay has for many years been engaged in the specialised treatment of cancer, with patients from all parts of India and other countries coming for treatment. In 1956 a new research institute, the Indian Cancer Research Centre, was built under the guidance of Professor V. R. Khonolkar, its first Director. Following his retirement in 1963 the hospital and centre became amalgamated, under the Department of Atomic Energy, as the Tata Memorial Centre under the directorship of Dr J. C. Paymaster. Particularly important during this time were the developments in cancer epidemiology. Dr D. J. Jussawalla became director in 1973; he too is interested in this field.

Cancer

The Indian communities, with their extremely rigid marital customs, still provide a unique field for the study of cancer epidemiology, but intermarriage may destroy all these possibilities within the next 50 years. Carefully planned activities are now, or soon will be, in operation; already a study is in progress in a rural area (Colaba) and others are in prospect. The chewing of Pan with tobacco is a major factor in oral cancer, but chewing, smoking, the bidi and dietary factors also lead to a high incidence in the pharynx, oesophagus and upper intestinal tract generally. The most valuable information is likely to be obtained from comparative studies of Indian migrant populations who have acquired new habits, particularly in Africa. Breast cancer is also common in India and epidemiological studies of the incidence in Parsi (late marriages) and Hindu women (who have early marriages) show a higher incidence among Parsis.

These epidemiological studies are being backed by collateral studies in carcinogenesis at similar sites. Of particular interest have been the studies in breast cancer. An inbred strain of mice (ICRC strain) has been developed which shows a high incidence of breast cancer and of leukaemia. Interesting relationships between B and C particles and the role of hormones in cancer incidence have been worked out with these mice. Ultrastructure studies are proceeding in human breast cancer and in the investigation of common base sequences in B particles and in the nuclear DNA of breast cancer patients.

A second major research theme at the centre is immunobiology. Studies in oral cancer have already shown evidence

for cellular immune response, particularly in patients with oral tumours subjected to electrical cauterisation.

The third theme now being embarked on is combination therapy, with special reference to cancers common in India. Under Dr Jussawalla, important advances have been made at the Centre in treating patients with a combination of chemotherapy, radiotherapy and surgery for various types of cancer. The centre has potential for developing new drugs from extracts of Indian natural products. A screening programme for plant products based on National Institutes of Health procedures has been set up using P388 and L1210 mouse tumours. Several active plant products have been isolated. The value of such studies probably lies in adding to the repertoire of drugs available for combination therapy. Systematic experimental work is being carried out on the characterisation of relevant human tumours. These include drug assays by organ or monolayer culture, basic cytology and, in particular, cell surface studies. An important advance has been made in isolating from cobra venom pure proteins which are not neurotoxins but which combine with specific sites in the surfaces of cells including tumour cells. Such agents are likely to be of value in the cytodagnosis of human cancers. Of very topical interest is the work on nuclear magnetic resonance. This approach shows promise as a diagnostic aid based on the spin lattice relaxation time of cell water.

Leprosy

Leprosy is in some ways a more pressing problem in South Asia than cancer. The number of leprosy cases in these areas totals several million and is on the increase. Theoretically, leprosy could be prevented by an intensive public health programme but in practice it may be many years before these programmes lead to a reduction in the number of cases, particularly in view of the population explosion. Dapsone treatment is effective, particularly in early cases, but requires daily doses for 5–10 years or even a lifetime in lepromatous cases. Infection continues to spread in the population at large despite fine work by devoted workers engaged in the care of leprosy patients.

Professor Antia, at the Tata Department of Plastic Surgery, has for a number of years been engaged in plastic surgery of leprosy patients. This is extremely important for rehabilitation, leprosy being almost as much a social problem (with its stigma) as a medical problem. He has also built up with the support of industrialists training centres where leprosy patients can learn new crafts and engage in active work in small factories. But the total number of workers in the field is still small.

A team is being built up for various studies, including pathology of the most suitable sites for biopsy of leprosy patients, the investigation of the localisation of bacillae in nerve cells and their effects on nerve conduction velocity. Teams work on the cell biology of leprosy, including the intracellular culture of the organism, and on the use of the mouse footpad. Developments in the general field of microbiology and cell biology may well prove valuable in the future for leprosy. Techniques for the rapid assessment of the viability and growth of the bacillae may lead to a deeper understanding of the metabolic requirements of the organism and hence to systematic research in leprosy chemotherapy (for example DOPA itself might prove to be a specific metabolite for *Mycobacterium leprae* (Prabakaran)) and the problem of treating intracellular bacillae as well as extra-cellular bacillae may now be investigated in living systems by autoradiographic methods so that the systematic study of human biopsy specimens is possible.

This account refers specifically to work in Bombay, with which I am familiar. In other parts of South Asia also, dedicated workers are actually engaged in the treatment of these two diseases and in research. The need is great because a large fraction of the world population lives in this region.

international news



The Pasteur Institute — independent but fading fast? Picture by Robin Laurance

AFTER nearly 90 years of unparalleled contributions to medical science, the Pasteur Institute in Paris is battling against a financial crisis and is in danger of closing. Set up with private money in 1888 for Pasteur to treat his rabies patients, the institute has remained stubbornly independent ever since. Over the years its researchers have won no less than eight Nobel prizes for medicine and have developed sera and vaccines for rabies, yellow fever, diphtheria, tetanus, tuberculosis and influenza. But despite this, and because of the escalating costs of research, the institute is running at a 15% deficit on its annual budget and observers say it could close down within five years.

The job of giving it a new lease of life falls largely on its director Jacques Monod, a musician turned biologist who decided there were "better chances for second rate biologists than for second rate orchestral conductors." But far from being second rate, Monod shared with two Pasteur colleagues a Nobel prize for medicine in 1965 for fundamental work on genetics; and in 1970 published *Chance and Necessity*, a philosophical treatise on the mechanics of inheritance, which was a best seller in France running second only to *Love Story*.

The headquarters of the institute in the Rue du Docteur Roux on Paris's Left Bank includes a 120-bed hospital for infectious diseases, a comprehensive science library and a total research staff of 1,150 including 300 postgraduate

Guiding the Pasteur through financial troubles

Robin Laurance, Paris

trainees. High specialisation is the keynote of the institute since it is orientated largely towards microbiology. Fifty laboratories provide facilities for basic and applied research into bacteriology, virology, physiopathology, immunology, molecular biology and other related studies.

Monod's office looks across to the original building where Pasteur lived and worked, and where doctors still treat rabies today in Pasteur's small book-lined consulting room. "One of our problems", says Monod, "is that we still live in the past. Members of the old institute fail to realise that we cannot maintain the position and style the institute enjoyed in the early part of the century."

One of the most severe financial handicaps has been the absence—until 10 years ago—of any patents on the institute's products. "If we had been collecting royalties all these years", says

Monod, "there would be no financial crisis." Now, Monod has to enforce the patent policy "even more ferociously." He has asked his colleagues not to publish a single word about new discoveries until a patent has been applied for. And it is a rule that non-commercially-minded scientists find hard to accept.

But even with royalties and licence fees, the institute's income was still not going to match its research needs. So last year Monod formed the production and sales side into a high powered commercially orientated organisation called Institut Pasteur Production (IPP) with all the shares held by the Pasteur Foundation. Monod became President and he spent nine months finding the right men to run it for him. He brought in Nestlé's head of home marketing as Vice-president and Michelin's sales chief to run the marketing division.

Monod is bitterly opposed to any suggestion of a government takeover and even less willing to see the institute absorbed into the university system. And he insists that he is ethically justified in enjoying the freedom from state or capitalist control. "Of course no French government could afford to watch the institute disappear because it carries such prestige—it's a national emblem. But if it did have to step in it would appoint the executive and effectively take control. Everything would get bogged down with typical French bureaucracy—the plague of this country of ours—and Pasteur would lose a great deal. And the universities are so badly

run, it would be even worse to come under their control." Monod has no hesitation in saying he would resign on the spot in the event of either fate.

A fund-raising campaign last year raised £2 million, and IPP's first year showed promising results with sales totalling £8 million, due largely to a new 'flu vaccine which looks like being more effective than anything else on the market. Two million shots have been sold since last October but it is still not available in the United Kingdom or in the United States as it has yet to satisfy the drug control authorities.

"I think the authorities have gone too far", says Monod. "The thalidomide disaster has made them overcautious and companies bigger than our own IPP are facing closure because the exhaustive trials are simply costing them too much. When I'm in a good mood, I think of the whole thing as a kind of sport. Maybe the odds at 2:1 in favour, and that makes it a game worth playing."

The institute's latest discovery, about to undergo clinical tests, is a vaccine which will give the body increased resistance to all bacterial and viral diseases. If proved successful it could be the discovery of the decade. But with IPP's distributor in the United Kingdom forced to offer it at such low prices for vaccines to compete favourably on the British market, it too may never be used in Britain.

"It is a mistake", points out Monod, "to believe that we have come to the end of vaccine discovery." He says that whooping cough vaccine is still far from satisfactory and that vaccines against viral hepatitis and against gonorrhoea are urgently needed. And it is these fields, and in immunology as a whole, that Monod believes the institute's researchers can, given the chance, make vital contributions. "But there's no doubt", he warns, "that there is still a very serious danger of the institute closing. If IPP is given time to grow—and we must treble the present sales—then we'll be all right. It all depends on how long that takes."

AEC case for fast breeders

Colin Norman, Washington

In June last year, the US Court of Appeals in Washington dealt a potentially severe blow to the United States fast breeder reactor programme—the great white hope for meeting the country's energy needs by the end of this century. The court ordered the US Atomic Energy Commission (AEC) to obey the laws of the land by publishing a comprehensive analysis of the combined environmental effects of several hundred breeder reactors scattered throughout the United States. The AEC has now produced a massive draft report which predictably argues that the reactor will be environmentally acceptable, safe and desperately needed.

The report, which runs to some 2,000 pages, is now doing the rounds of other government agencies, public interest groups and environmentalist organisations, and it will be the subject of a public hearing on April 24. Not surprisingly, it is generating a good deal of heat in the long and bitter battle between the Atomic Energy Commission and its critics over the safety and acceptability of nuclear power.

The objective of the court suit, which was brought by the Scientists' Institute for Public Information, was to force public discussion of the dangers and environmental consequences of the entire commercial breeder reactor programme, before development had progressed so far that it would be difficult to halt. The critics reckoned that if the huge scale of the proposed programme, together with the dangers inherent in fuel processing and waste disposal, could be brought into the public spotlight, the case against the breeder would be compelling.

But the AEC, helped by the energy crisis in general and by the Arab oil boycott in particular, has turned the argument about the size of the programme around and thrown it back in

the faces of its critics. The agency argues, in short, that the breeder reactor will offset United States dependence on imported fuel, that it provides the surest and most acceptable means of meeting the predicted demand for energy in the 1990s and beyond, and that to abandon breeder reactor development now could put the United States in the position of relying on foreign breeder technology at some time in the future.

First, the scale of the programme. The AEC's report indicates that if all goes according to plan, the first commercial breeder reactor will start operating in 1987 and by the year 2000, 400 individual plants, each capable of producing 1,000 MW of electricity will be at work in the United States. They would be producing between them as much electricity as the entire United States electric power industry generated last year. By the year 2020, as much as 2.2 million MW could be generated by fast breeder reactors.

The AEC argues that some other means of providing that energy must be found if the breeder reactor programme is halted and that there is nothing on the horizon which is either capable of filling the gap or technologically proven. To which the critics reply that if the money to be spent on breeder reactors were put into other energy systems, such as solar power and geothermal energy, then they could be developed to the point where they would be capable of meeting energy demands. The AEC replies that if it turns out that the concepts, promising as they are, cannot be turned into energy in time, the United States would find itself in an energy crunch by the end of this century.

What particularly worries opponents of the breeder reactor is the amount of plutonium which will be produced by a string of reactors operating around the country. The advantage of the breeder over conventional nuclear power plants is that it can be fueled on nonfissionable uranium which cannot be used in the so-called first generation reactors. The uranium is converted during the reactor operation into plutonium, which can

Dounreay — Britain's first fast breeder station, on line without objections



then be used to fuel the reactor on another operating cycle—in other words, the reactor breeds its own fuel.

The advantage is that the breeder reactor does not require additional uranium to be mined, which is fortunate because uranium reserves in the United States are estimated to last for only another 25–30 years. But the problem is that plutonium is highly toxic, its radioactivity decays extremely slowly and it can be turned relatively easily into the heart of a crude atomic weapon. Opponents of the breeder programme have therefore concentrated their attacks on the health and national security hazards of plutonium. Clearly, the larger the commercial breeder programme, the more plutonium will be produced, and the greater the perceived hazards.

The AEC analysis states, however, that strict safeguards are enforced at all facilities which handle plutonium, to prevent any of it from falling into the hands of people looking for a nuclear weapon. Unfortunately, however, a study conducted last year by the General Accounting Office (GAO) concluded that the safeguards at some installations would scarcely deter an amateur burglar, let alone a well planned theft. Although the AEC maintains that it has stepped up its safeguards in the light of the GAO's findings, the public relations aspects of the matter are difficult to overcome.

As for the health risks, the AEC's draft report concludes that the entire breeder reactor programme is likely to cause less than 2.2 cancer deaths in the United States over the whole lifetime of the reactors, fuel fabrication plants and waste disposal facilities. But that conclusion was challenged even before it appeared in print by two nuclear energy specialists working for an environmental organisation called the Natural Resources Defense Council and it is likely to develop into a bitter wrangle.

The two critics of the AEC's analysis, Dr Arthur Tamplin, an ex-AEC employee and long a thorn in the agency's flesh, and Dr Thomas Cochran, have challenged the basis of the nuclear industry's estimates of the potential damage caused to the lungs by particles of plutonium. They have argued, in short, that it is unrealistic to base safety standards on the likely dose of radioactivity to the entire lung from tiny particles of plutonium. A single particle is likely to give a dose of radioactivity to the whole lung of about 0.003 rem per year but Tamplin and Cochran argue that the tissue immediately surrounding a particle of plutonium will get a dose of radioactivity as high as 4,000 rem per year. Therefore, they argue, the AEC should reduce the amount of plutonium allowed

to be released into the atmosphere by a factor of 115,000.

Those two points the potential for diverting plutonium into the hands of somebody capable of producing a crude bomb and the health risks from plutonium released into the atmosphere—are thus likely to form the centrepiece of the public hearings next month. But environmental causes have been taking a battering in the past few months in the United States, as public attention has swung away from the environmental crisis to the more obvious effects of the energy crisis, and the AEC can be expected to play the breeder reactor's promise of supplying 2.2 million MW of electricity for all it's worth.

It is, perhaps, worth noting that earlier this month, the British 250 MW Prototype Fast Reactor at Dounreay became critical, and the fast breeder reactor programme is proceeding in Britain relatively unencumbered by requirements for public discussion of risks and benefits.

Nuclear inquiry

WITHIN weeks of the Dounreay Prototype Reactor going critical, and while the choice of Britain's next nuclear reactor system hangs in the balance, the Royal Commission on Environmental Pollution has announced its decision to investigate standards of nuclear safety in the country. The Commission on Environmental Pollution is a standing commission, recently reconstituted under the chairmanship of Sir Brian Flowers, and the choice of subject for its first inquiry is thought to have been decided by the long term implications of a switch from conventional to nuclear power generation rather than by the immediate crisis of consciences over hardware selection.

Sir Brian has taken pains to point out that members of the commission "do not feel able to involve themselves in highly technical questions of reactor design in relation to safety, or with comparisons between one type of reactor and another". Nevertheless, in the next breath he added that in the general grounds of concern about environmental hazards "in a choice between reactor systems great and perhaps overriding weight should be given at the present time to the issue of safety". The commission will also examine the problems of radioactive waste disposal and the transport and storage of nuclear materials, particularly plutonium.

Business report

Roger Woodham

ALBRIGHT and Wilson, the chemical company, has had to reduce the book value of this ill-fated phosphorus plant at Long Harbour, Newfoundland, because of continuing production difficulties. In 1973 the plant, designed to produce 72,000 tons of phosphorus a year, only had an output of 40,000 tons, which, at that, was an improvement on the situation in 1971 and 1972.

The Long Harbour plant is one of only four of its size in the world—it consumes 140 MW of electricity—and Albright and Wilson admitted in 1971 that the two manufacturers using a comparable electrode arrangement in the furnace had experienced and overcome similar difficulties—broken electrodes. Nonetheless these problems, which have necessitated several major shutdowns, do not seem to have been sorted out at Long Harbour and the company is throwing in at least part of the towel by revaluing the plant from £19.9 million to £11.2 million.

In spite of these upsets Albright and Wilson managed to notch up an increase in profits from £2.75 million to £7.47 million, just short of its 1964 best of £7.57 million.

With prices of raw materials as they are, however, the company's future fortunes are going to depend on how one of its major customers, the farmer, reacts to the prospect of higher phosphate prices.

● The prices in Britain of scientific instruments are by no means all susceptible to the ravages of inflation, although the cost of an instrument that pushes a technique to the limit possible at a given time does jump ahead in leaps and bounds.

Instrument price trends

	Routine	Research
NMR	£10,000 (£10,000)	£40,000 (£80,000+)
IR	£1,410 (£1,420)	£8,000 (£20,000)
UV	£700 (£750)	£5,000 (£8,000)
GC	£600 (£1,000)	£2,000 (£3,000)

Broadly speaking, the prices of routine instruments that have the same capabilities as their counterparts ten years ago, say, have marked time and in real terms have fallen. The reason is that companies have made use of new technology to simplify and cheapen the manufacturing process. With really high performance equipment, on the other hand, the manufacturer is involved in much larger development costs and is much more sensitive to the effects of wage inflation. Top notch instruments

are thus much more expensive now than they were ten years ago, a situation which is often described in terms of a 'sophistication factor'. In the case of imported instruments, which account for a greater proportion of the research type than the routine type in Britain, the customer also, of course, has to contend with price changes introduced by fluctuating exchange rates.

The table shows the state of affairs for nuclear magnetic resonance (NMR), infrared (IR) and ultraviolet (UV) spectroscopy and for gas chromatography (GC). The prices in brackets are the prices today and the others are the prices ten years ago.

Oil on troubled waters

A SYMPOSIUM on hydrocarbons in the marine environment has been postponed by the Ministry of Agriculture because they do not think the meeting is necessary. The organisers, the Torry Research Institute and the Marine Research Station at Aberdeen, planned to discuss the effects of oil pollution at Aberdeen in May. About 100 scientists said they would attend, but they are now being told that the symposium has been postponed. Apparently, somebody who should have been asked wasn't. The Ministry of Agriculture's Administrative Officer, Mr W. R. Small, explained that the symposium is not necessary because there are "six or seven" other symposia going on which deal with the same, or allied, subjects. "This should never have been mooted in the first place without the right people being asked",

he thought. "There is simply no unanimity about the urgency of the symposium at this stage. There is a limited corpus of scientists scurrying around these meetings, and at this moment the ministry cannot afford to have its resources spent unnecessarily. You know, it's like a *Beau Geste* fort, with the same men appearing at different battlements all the time."

A corollary to this spirited assessment of the marine biologist's round is that the men who appear at the battlements on home ground tend to include a fair proportion of junior scientists who are actually engaged in research in the field. The overseas junkets are sometimes made up of more senior men who count foreign travel among their hobbies. The Aberdeen cancellation note points out that the symposium would have clashed with a similar meeting in Maryland—a conjunction which might have caused a crisis of conscience among the senior men, but would not have presented a problem to the junior workers who would have been glad of the chance to talk about pollution problems on their own doorsteps.

Furthermore, Aberdeen is not doing at all badly for profits from services related to the offshore oil industry at the moment and some people would not really be interested to hear about hydrocarbon pollution if oil ran ankle deep in the streets. The Scottish press is keen to pick on stories involving damage to the environment and would almost certainly have been looking for pickings at the Aberdeen symposium. There was a chance that the benefactions of the oil industry would be called into question. This has now been avoided.

More planning problems at Loch Carron

THE public inquiry into setting up deep-sea oil platform construction sites at Drumbuie, Loch Carron on the west coast of Scotland opened again on March 18 after being adjourned for several weeks, but was immediately adjourned again at the request of the National Trust for Scotland, the main objector. The trust was opposed to an entirely unexpected environmental report being placed as evidence.

The trust owns the land on which the platforms will be built if the Drumbuie site is approved for development. They were objecting to a surprise environmental report commissioned by the Scottish Development Department from Sphere Environment Consultants of London. This report considered several other possible sites in the area. None of the objectors had been told that the report was in preparation and their counsel successfully moved for an adjournment until April 1.

The National Trust and the other ob-

jectors had finished giving their evidence against the development when the inquiry was adjourned in February, and this new development will considerably prolong matters. The environmental report and an expected consulting engineer's report, commissioned by the Department of Energy, both come out in favour of Drumbuie as opposed to other sites in the area but give alternatives if the National Trust land does not become available. As the proposed bill to nationalise land needed for oil platform construction is not going ahead under the new Labour government, a special bill in Parliament will be needed to use the trust's land.

Sphere suggested a site on the Isle of Skye near Broadford Bay as their second choice. On the other hand the consulting engineer's report gives a site on Loch Kishorn, near Carron, as the second choice and Broadford Bay as the third.

Australian science news

Peter Pockley, Sydney

IN a speech to the Science and Industry Forum of the Australian Academy of Science in February, Dr Moss Cass, the Minister for the Environment and Conservation, announced the formation of a new Bureau of Environmental Studies within his department. In doing so, Dr Cass has rejected alternative proposals for establishing a statutory institute, or for strengthening the existing responsibility for some environmental research within the Commonwealth Scientific and Industrial Research Organisation (CSIRO), which in any case comes under the Minister for Science, thus revealing the old problem of duplication where departments overlap.

The Bureau of Environmental Studies will have three main areas of responsibility. First, it will survey, assess and integrate existing studies done by other groups. Second, it will develop programmes to fill identifiable gaps. A third group in the bureau will try its hand at environmental 'futurology', with the aim of anticipating the problems of 20 to 30 years ahead. Here we see the strong strand of anti-industrial growth and humanism in Dr Cass's philosophy, for this group will be charged with developing some kind of human progress index. For this, Dr Cass suggests the term 'gross domestic welfare', which he hopes could be derived from a combination of measures of environmental quality, social indicators and productive capacity in both goods and services. Recruitment for the bureau's staff will start shortly. It looks as though they will need to be in the market for social philosophers as much as ecologists.

● In a further move indicating the diplomatic initiatives between the Australian Labor government and the communist world, the first exchange of visits between Australian and Chinese scientists was announced recently. A party of nine Australians started an 18-day visit to Canton, Peking, Shanghai and other centres on March 18. The party of four representatives of the Australian Academy of Science, four from the Australian National University (ANU), and one Asian scholar as interpreter will be led jointly by Professor Sir Rutherford Robertson, President of the Academy, and Dr R. M. Williams, Vice-Chancellor of ANU. The inclusion of an interpreter chosen by Australia is seen as a significant advantage for the party; he is Professor Liu Ts-un-yan, Head of the Department of Chinese at the ANU.

The scientific interests of the Australians include astronomy, spectroscopy, plant physiology, genetics, mathematical



In Britain the Family Planning Association (FPA) has taken over the production of a comic about birth control methods, called *Too Great a Risk*, which was the brainchild of the FPA's South-west London branch. The headquarters of the FPA took control of production and distribution because the comic proved so successful on a local level: since its launch in March last year 70,000 copies of *Too Great a Risk* have been supplied to school and college teachers, youth

leaders, social workers, probation officers and doctors. Eleven countries have ordered copies, Holland and West Germany have acquired second copyright for home production, and requests for supplies come in at an ever increasing rate at home and abroad. The comic pinpoints the dangers which a boy and girl can face through ignorance of birth control. It costs 2p plus postage, or £2 a hundred, including postage, from FPA headquarters at 27-35 Mortimer Street, London.

statistics, classical thermodynamics, nature conservation, biomedical sciences and civil navigational aids. The party will officially be guests of the Academia Sinica, who will sponsor a return visit of Chinese scientists to Australia later in the year.

● Only a few hours before rushing back to Britain to preside over the general election, Queen Elizabeth performed her first major constitutional function in her newly designated role as 'Queen of Australia'. This was the formal opening of the 28th Federal Parliament in Canberra. Despite the governing Labor leaders' avowed preference for a republic in Australia in due time, this occasion was marked by as much ceremony and circumstance as in the "mother parliament" at Westminster.

The Queen's speech at the opening of Parliament on February 28 revealed, in tersely abbreviated terms, a massive programme of legislation which Prime Minister Whitlam's government hopes to push through in its second year in

office. (Some of it will truly require much hoping and pushing because the government faces a hostile opposition in the Senate where the opposition parties hold a firm majority—a Senate election due in the middle of this year is unlikely to change the balance of power in this upper house.)

Science *per se* rated a mere six words, namely "A science council is being planned". This passing mention confirmed how tentative are the current plans of the Minister for Science, Mr Bill Morrison, for replacing the former Advisory Committee for Science and Technology which he axed a year ago. Mr Morrison has said that legislative action is needed to establish the proposed Australian Science Council on a proper footing but hopes are now dimming that his portfolio has enough political pull to get some priority for science affairs on the legislative list. It now looks unlikely that a Science Council could be formed before next year, the last scheduled year of Labor's

three-year term.

Previous speculation that Mr Morrison would be promoted out of the science portfolio is now dead. He has shed all but residual responsibility for Papua New Guinea affairs and there is a fair flow of publicity over the minister's name, relating mostly to administrative changes within the minister's responsibility. But the politics of science in Australia are rating no mention at all from the news journalists, the commentators and, worse, from the politicians themselves.

● Postscript to our news (*Nature*, 247, 500; 1974) of the appointment of Professor E. Joseph Wampler as Director of the Anglo-Australian Telescope. His salary has now been disclosed as \$A23,000. This is \$A3,850 more than that of the new Director of the Australian Institute of Marine Science (which is the equivalent to the salary of a full professor) and is within the range of salaries paid to Chiefs of CSIRO Divisions.

correspondence

Academics in Chile

SIR,—The international scientific community must be aware of the dramatic condition of the Chilean academic community. The military authorities who have taken over the power in all Chilean universities issued new rules stipulating that:

"Any nomination or contract must from now on be considered strictly provisional. The Special Commission [installed by the military rectors] will propose renewals or cancellations of such contracts in each particular case.

All students must apply for reinscription . . . The Special Commission will decide on the opportunity of accepting or refusing such applications.

Any professor, administrative employee, technician or student suspected by civil or military courts will be immediately suspended . . . Any one of them being sentenced will be dismissed; students will be permanently expelled from the Universities.

The same action will be taken towards those having answered citations from the courts . . ."

This quotation is taken from an official statement issued by the new authorities in charge of one Chilean university on September 28, 1973. Analogous decisions have been made in all universities, including the Universidad Católica de Chile. As a consequence of this, many professors or academic staff have lost their jobs. In the most favourable cases (such as that of professors on official leave or absence abroad), scientists "have been granted permission to present their resignation" (usually with retroactive effect). Many have been arrested, on an arbitrary charge or no charge at all, usually after anonymous denunciations of neighbours or colleagues. Many of them are still in prison and an official International Lawyer's Commission has presented evidence for many cases of physical torture. In La Serena, professors against whom no special charge could be found have been executed in spite of having been condemned to minor sentences (less than two months of imprisonment).

Several other members of the academic community have been executed without being permitted to present their defence.

In various universities, departments like geography, sociology, economics and biophysics have been completely dismantled.

These facts must be publicised and

scientists all over the world should press governments and international organisations in order to prevent further repressive actions. In addition, there is an urgent need for help to academic people who already could, or will eventually be able to, leave the country. We have on record many excellent applications in all fields ranging from mathematics to social sciences.

All those able to offer laboratory space and positions, even on a temporary basis, should contact the Committee of Assistance to Chilean Scientists (CACS), by writing to M. Imbert, Collège de France, 75231 Paris-Cedex 5.

Yours faithfully,

H. M. GERSCHENFELD

*Ecole Normale Supérieure,
Paris, France*

F. GROS

F. JACOB

A. LWOFF

J. P. CHANGEUX

Institut Pasteur, Paris, France

MASLE

*University of Copenhagen,
Copenhagen, Denmark*

P. WALL

*University College,
London, UK*

North Sea oil

SIR,—Although I agree with the opening paragraph of Eleanor Lawrence's article on the planning aspects of North Sea oil (*Nature*, 247, 416; 1974) I feel that the remainder of the article leaves much to be disputed.

What does Mrs Lawrence mean by 'getting the oil ashore as quickly as possible'? If she means seeing the oil flow as soon as possible then there is probably nobody against it. Already, nine platforms are in various stages of construction for the Auk, Beryl, Brent and Forties fields, and at least one or two other fields will be exploited using drilling rigs rather than production platforms. There is, therefore, no block to how quickly the first oil will come ashore. If, however, she means by 'the most extreme preservationists' those who are opposing the intended maximum rate of exploitation of this important resource (as is current policy) then she is referring to no splinter group of cranks. It includes Liberals, Scottish Nationalists and a good number of Socialists who have all committed themselves against seeing oil used in this way.

It is, I would agree, right to criticise present planning procedures because

they treat each separate proposal in isolation rather than in the context of a coordinated plan. This is plainly ridiculous. The advantage that the present system offers, however, is time to reflect. The technological situation in relation to oil extraction from the sea bed is changing so rapidly that there are real dangers that what seems essential today will be of historic interest in a couple of years' time. Thus although the Condeep gravity concrete structure is currently in favour (and if to be built in Britain, probably needs to be constructed at Loch Carron) this ignores the alternative deep-water platform designs already being offered by different companies at existing yards, ignores the recent innovations in 'floating' platforms announced recently and ignores the technology for seabed completions which it is expected will be erected for deep water cases within the next two years. It would be a tragedy if rushed decisions caused the destruction of a way of life at Loch Carron, especially if it were for only a short term return.

The real iniquity of the present system is that the objectors should be involved in costs of many thousands of pounds. This is not because of the law itself but because of government choice. Section 267, subsections 7 and 8 of the Town and Country Planning (Scotland) Act 1972 states clearly that costs may be awarded to the objectors but the Secretary of State for Scotland has consistently refused to consider doing this. Thus, although objectors must pay their costs from their earned and taxed incomes, development companies can offset their costs against profits before tax. At Dunnet Bay the objectors were faced with a bill of £3,000 while Chicago Bridge decided to go elsewhere. At Loch Broom, the Action Group incurred costs in setting up its case against the proposed development at Ullapool before Mowlem discovered that the site was unsuitable anyway. Meanwhile the taxpayer contributed to an environmental impact survey that was never needed. The main objectors at Drumbuie will be faced with bills of tens of thousands of pounds. There is a very clear case indeed, in the light of the special circumstances that oil developments present, for the proposing companies to be held responsible for costs of genuinely concerned objectors.

TERENCE W. HEGARTY

*North Sea Oil Coalition,
2 Tayside,
West Ferry,
Dundee DD5 1DW, UK*

news and views

Genetic factors in hepatitis

ALTHOUGH much has been written about the possible mechanisms leading to the establishment of the chronic carrier state of hepatitis B antigen and the remarkable geographical variation in the prevalence of such carriers, which may number in some areas many hundreds of thousands, the genetic hypothesis promulgated by Blumberg and associates (*Proc. natn. Acad. Sci. U.S.A.*, **62**, 1108; 1969) remains a contentious issue among students of viral hepatitis.

Examples of inherited susceptibility to infection have been described in several animal species including man and it is recognised, of course, that many other factors in the environment such as sex, age at exposure, state of nutrition, immunological factors, socio-economic circumstances and so on also affect susceptibility and resistance to infection. The discovery of hepatitis B antigen resulted from the interest of Blumberg and associates in inherited polymorphisms. Family studies carried out in the islands of Cebu and Bougainville in the Pacific, and on a more limited scale elsewhere, seemed to be consistent with the hypothesis that a gene designated *Auⁱ* is responsible for persistence of hepatitis B antigen once infection with hepatitis B virus has occurred. This gene is considered to be common in tropical areas but rare in temperate zones. Individuals homozygous for this gene (phenotype *Auⁱ/Auⁱ*) would have persistent antigen without overt manifestation of hepatitis, although they may remain as carriers of the associated infectious agent, whereas the heterozygotes (phenotype *Auⁱ/Au*) and individuals without the gene (*Au⁰/Au⁰*) would not remain as carriers. Persistence of hepatitis B antigen in certain other diseases such as leukaemia, lepromatous leprosy and Down's syndrome (mongolism) is believed to be of a different nature, namely an inherited susceptibility to infection not only with hepatitis B but with the agents responsible for these other diseases (Blumberg, *Ann. N.Y. Acad. Sci.*, **197**, 152; 1972). This would explain the association of the antigen with other diseases in circumstances in which the individuals with the susceptibility gene were exposed to hepatitis B virus; and absence of the association when exposure did not occur.

Ceppellini and colleagues (Convegno Farmitalia, *Antigene Australia ed Epatite Virale*, Minerva Medica, Torino, 53; 1970) also studied the segregation of the postulated trait for persistence of hepatitis B antigen among 165 families in Sardinia. A similar conclusion on the presence of an autosomal recessive locus was reached, although with some distinct qualifications. First, there was a sharp decline in the carrier rate of the antigen after the age of 20, an observation which has also been reported from many other high prevalence areas. Second, all of the reported matings, excepting one family, were between parents where only one parent was antigen-positive or where both parents were negative. In the family in which the antigen was detected in both parents, only two of the seven children were also positive. This finding on its own would rule out a simple hypothesis of recessive inheritance with complete penetrance of the homozygous recessive genotype. Ceppellini considered this fact, but decided to disregard the critical mating.

Now Vyas has reported (*Nature*, **248**, 159; 1974) a second critical mating which also fails to support the postulated inherited susceptibility to persistence of hepatitis B antigen. A pedigree with antigen-carrying parents and their progeny of three children were tested for hepatitis B antigen and hepatitis B antibody by several methods including the most sensitive techniques currently available—radioimmunoassay and passive haemagglutination. The antigen was not found by repeated testing of the serum of the three children. Hepatitis B antibody was, however, detected in the serum of the eldest son, aged 10 years, and so provides unequivocal evidence of past exposure to the antigen resulting in a normal immune response to infection. This is crucial evidence against susceptibility to the chronic carrier state inherited as a simple autosomal recessive trait.

Lederberg (*Hepatitis and Blood Transfusion*, edit. by Vyas, Perkins and Schmid, Grune and Stratton, New York, 89; 1972) previously summarised the position by stressing the alternative hypothesis to the genetic factor—that familial clustering may be entirely a function of an increased opportunity for environmental exposure to the virus. Furthermore, it stands to reason that if a parent is excreting the virus this will augment the chance that any of the offspring may acquire it, particularly if the mother is the transmitter.

Another aspect of the genetic basis of susceptibility to hepatitis is implicit in the finding that the frequency of certain genetic markers on the heavy chains of immunoglobulin G (Gm types) was always greater in multiply-transfused Italian patients with thalassaemia who were persistent carriers of hepatitis B antigen than in those with hepatitis B antibody (Blumberg and associates, *Nature*, **236**, 28; 1972). With respect to Gm types, individuals who are heterozygous for Gm factors are less likely to encounter Gm types different from their own and are therefore less likely to develop anti-Gm. Such persons when infected with hepatitis B virus, which incorporates certain host components in its protein coat, would be likely to develop persistent antigenaemia and minimal liver damage. On the other hand, individuals who are homozygous for Gm types (that is, they have fewer Gm types) are more likely to encounter Gm types different from their own and thus develop antibodies to them. Such persons, when infected with hepatitis B virus, would be more likely to have an acute infection and an increased probability of developing hepatitis B antibody. Schanfield and colleagues (*Nature new Biol.*, **243**, 81; 1973) examined for Gm allotypic markers samples from blood donors submitted routinely for testing for hepatitis B antigen in San Francisco and Minneapolis. The frequency of the various Gm phenotypes did not vary significantly among the Caucasian donors investigated according to the presence or absence of hepatitis B antigen or its antibody. Similarly, there was no significant excess of anti-Gm among individuals with hepatitis B antibody when compared with those with persistent hepatitis B antigenaemia. These findings are not in accord, at least in a normal adult population, with the hypothesis of a direct association between the polymorphisms of the Gm types and hepatitis B antigen.

It does not follow, of course, that the hypothesis of a genetic predisposition to hepatitis did not have a productive impact, since it did generate much discussion and considerable experimentation.

A. J. Z.

Blue-green algae as model organisms

SOME filamentous blue-green algae show a one-dimensional pattern of differentiated cells. In *Anabaena* and *Nostoc*, heterocysts arise from vegetative cells. Heterocysts are relatively large, rounded, refractile cells, which are believed to fix nitrogen. The transformation from vegetative cells to heterocysts by way of the intermediate proheterocysts can be stimulated by transferring algal filaments to a medium free of fixed nitrogen. The conditions required vary among the species; for example, in *N. muscorum* and *A. cylindrica* there is not a well-defined pattern of proheterocysts in a medium containing fixed nitrogen, although there may be in *A. catenula*.

Two separate problems have recently been addressed in the literature: first, how the pattern of heterocysts is controlled; and, second, what the function of heterocysts is and whether this can be correlated with the appearance of specific proteins during heterocyst differentiation.

Mitchison and Wilcox (*Nature*, **239**, 110-11; 1972) found that vegetative cells in *A. catenula* divide with a mean time of 14 h. The best defined event in the cycle is the formation of a septum between daughter cells which takes 1-2 h. The divisions are always asymmetric and follow a simple rule which had no exceptions in the 600 cases observed. If a cell arises as, say, the left daughter of a division then its left daughter is always the smaller. Furthermore, heterocysts (which do not divide) only develop from the small daughter cells, which themselves grow more slowly than the large daughters although both attain the same size before their next division. This provides a very simple example of determination following a cell lineage rule, as if the vegetative cells were behaving as a set of stem cells. Mitchison and Wilcox, however, say that they know of no other example in which this recursive rule is followed.

The spacing of heterocysts, although it depends partially on cell lineage, is finally determined by other factors, as most small daughter cells do not develop into heterocysts. Wilcox, Mitchison and Smith have examined these in detail (*J. Cell Sci.*, **12**, 707-723 and **13**, 637-649; 1973). In the first paper they define the pattern and make a model of the spacing mechanism; in the second they test the model experimentally, and report on the ultrastructural aspects of heterocyst differentiation.

In both species of *Anabaena* used the pattern of heterocysts is quite regular: in *A. cylindrica* there are 9.3 ± 2.8 vegetative cells between heterocysts (or preheterocysts) and in *A. catenula* there are 10.1 ± 2.5 . The simplest model to account for this was put forward by Fogg, who suggested that heterocysts produce a diffusible metabolite which inhibits further heterocyst differentiation. That is, there is a threshold concentration of inhibitor below which small daughter cells will differentiate into heterocysts. Wilcox *et al.*, however, feel that this model is too simple as they found that early differentiation towards heterocysts is reversible implying that there might be a competitive interaction as well.

In particular, the authors examined heterocysts that arose in the terminal intervals of filaments. In central intervals proheterocysts tend to appear exactly at their centre, but in the terminal intervals proheterocysts, and thus heterocysts, are displaced towards the end of the interval. On the simple threshold model one would expect to see heterocysts at the ends of terminal intervals, where inhibitor concentration would presumably be lowest. Wilcox *et al.* suggest instead, however, that an end behaves as though it were one half of a hypothetical interval consisting of the original terminal interval joined to its mirror image. That is, the end wall of the terminal cells as a reflecting barrier to inhibitor diffusion.

A terminal heterocyst would thus be equivalent to one half of an adjacent heterocyst pair, which almost never occurs. The addition of a competitive interaction to the simple threshold model could then account for the rarity of terminal heterocysts.

Wilcox *et al.* therefore made artificial ends by breaking *A. catenula* filaments in intervals where central heterocysts would normally be expected to arise. As expected, the developing heterocyst was displaced towards the break, producing a normal end interval within 8-12 h, a time too short for differential division to be important. They then, in *A. cylindrica*, made mirror-image heterocysts by breaking a cell close to a proheterocyst. As expected this led to regression of the proheterocysts, with a probability depending on the number of cells left intact between it and the break, although there are relatively few regressions when the cell next to a proheterocyst is broken. This may be because the proheterocyst forms a normal, permeable cell junction on the break side which could allow a leakage of inhibitor.

A further piece of evidence for compensation is that groups of proheterocysts may appear in *A. catenula*. All but in a group one regress, unless there are more than three cells in the group, when one heterocyst may differentiate at either end.

Wilcox *et al.* propose that even after a proheterocyst has begun to produce inhibitor it remains susceptible to its effects. When a threshold, which increases with developmental age, is exceeded, the cell regresses. The contribution of heterocysts to inhibitor production sum, so one can inhibit another. The most important feature, the competition, implies also that a heterocyst can inhibit its own development, as in regression after filament breakage.

In their second paper Wilcox *et al.* give further support to their model by investigating the conditions in which a heterocyst can inhibit itself. They used *A. catenula* and made filament fragments containing heterocysts at all stages of development with no, one, two or three neighbouring cells on each side. Regression frequency decreased both with developmental age and with number of neighbours. Moreover, heterocysts at the end of a fragment had a lower probability of regressing than heterocysts with a neighbour on each side, probably because inhibitor can leak through the heterocyst wall.

The competitive interaction which is enhanced by NH_4^+ can apparently be broken down by 7-azatryptophan (Mitchison and Wilcox, *Nature new Biol.*, **246**, 229-233; 1973). When it is added to the growth medium, at a concentration of $5 \times 10^{-6} \text{M}$ or greater, the spacing between heterocysts is reduced and multiple heterocysts may form. Alteration of the pattern begins within 10 h and is independent of concentration, although the final pattern is not. All the changes observed correspond to an increase of inhibitor level in their model, although there is no evidence to link the effect of 7-azatryptophan with any aspect of metabolism.

Fleming and Haselkorn (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 2727-2731; 1973) separated heterocysts from vegetative cells, in *Nostoc muscorum*, by treatment of the filaments with lysozyme and then centrifugation. They found that the increase of nitrogenase activity, which occurs on transfer of filaments to a medium free of fixed nitrogen, parallels the increase of number of heterocysts formed, and that heterocysts synthesise many proteins not found in vegetative cells. Furthermore, they were able to isolate (on SDS gels) three proteins which bore a strong resemblance to the structural proteins of bacterial nitrogenase the induction of which was also related to the increase in nitrogenase activity.

The authors speculate that heterocyst differentiation begins when the concentration of NH_4^+ falls below a threshold and that products of nitrogen fixation in heterocysts repress the development of, and nitrogenase synthesis in, neighbouring vegetative cells. As the ability to inhibit

further heterocyst development is acquired before pro-heterocysts can fix nitrogen, the authors suggest that pro-heterocysts might be able to transport NH_4^+ to their neighbours. They also point out that heterocysts must become refractory to repression as they continue to synthesise the structural proteins of nitrogenase while fixing nitrogen. Although there are many ways in which induction and repression might be mediated, it is interesting that both groups working on this problem have been forced to models with very similar qualitative features.

The blue-green algae proved a very simple system for the study of patterned differentiation. It should be possible not only to discover the mechanism for the control of differentiation, but also to correlate differentiation with events at the molecular level, a normally elusive ideal for developmental biologists. *Anabaena* and *Nostoc* thus join *Hydra* and *Dictyostelium discoideum* as model organisms in which the outlines of a developmental control system are known, and which are simple enough that the details of differentiation during pattern formation can be followed.

From a Correspondent

A Chinese view of tectonics

PROFESSOR J. S. LEE was a Chinese earth scientist highly regarded both in China and the West, so the appearance of a long posthumous article by him entitled "Crustal Structure and Crustal Movement" in *Scientia Sinica* (16, 519-559; 1973) is an event of great interest. The paper was written in 1970, and so cannot claim to be an ideal representation of present-day Chinese thinking on the subject, but it is nevertheless a most fascinating insight into Chinese approaches to tectonics; the more so because Lee's conclusions, based primarily on Chinese evidence, are simultaneously near to and far from Western views.

In a subject as explosively developing as the earth sciences, it is desirable to know how much of the literature of other than Chinese origin the author has consulted. It is clear even in this reference-free paper that Professor Lee had access to nothing beyond about 1963. The magnetic stripes off western North America are described in some detail, but what they imply "is not yet clearly known" beyond that basic or ultrabasic rocks are present. Mid-ocean ridge magnetic anomalies are not mentioned. Seismicity only receives the briefest qualitative attention. With this crippling lack of the ingredients that went into plate tectonics (and without the 1961 seafloor spreading hypothesis), Lee's synthesis is bound to be incomplete. He further complains that "reactionary imperialistic blocs" in scrambling for the wealth of the sea floor and other purposes have not published most of their data. Nevertheless his paper has much interest.

He first looks at the various hypotheses of crustal movement. Some geologists—most of the "traditionalist Americans and some orthodox geotectonists in the Soviet Union" (Belousov, no doubt)—insist that vertical movement is dominant and flatly deny the importance of horizontal movement. This he sees as founded in ideas of continental fixation and thus "metaphysical, and utterly dogmatic. This guiding thought was propagated to our land and has brought considerable loss to our geological cause". Lee then goes on to consider possible aspects of crustal movement—cooling and contraction, tides, expansion, convection currents (not ruled out but a "purely metaphysical hypothesis"), isostatic compensation, continental drift and what is called "China's own road". Most of these are dismissed peremptorily, but continental drift (separate from convection currents) is considered briefly as "not entirely unreasonable". Lee, however, cares little for palaeomagnetism and does not accept field reversals.

China's "own road" is an approach which he, as author of a textbook on the geology of China, finds most appealing: learn what you can from China's "extremely superior conditions of structural development". Extensive field work is necessary on structural theories—as Chairman Mao has it, "Man's knowledge is verified only when he achieves the anticipated results": advice perhaps more suited to debugging computer programs than scientific research. Once Lee discovered certain broad scale structural regularities in China, he was prepared to extend them to the rest of the Earth. These features are:

- A latitudinal system of E-W zones revealed by both topography and aeromagnetism. One is at 40° to 42°N and is seen to extend through Central Asia, Turkey, the Mediterranean, under the Atlantic, intersecting the Appalachians, producing an east-west gravity anomaly in the western United States and running into the Mendocino fracture zone. Other world circling zones at 32° – 34°N and 25°N are described and one or two in southern latitudes.
- A meridional system is identified by north-south trends, less obvious in China but strong elsewhere such as in the Urals and East Africa.
- The Neocathaysian system striking NNE-SSW is a feature of importance on the oceanic margins of Asia.
- The Cathaysian (NE-SW) system is again an Asiatic feature.

- Shear structural systems are complex but all reflect areas in which there has been shear in the horizontal plane.

Lee observes that surface features are often not in complete agreement with those at greater depth, "in other words they are detached from each other". Furthermore the upper part of continents is inconsistent with the ocean floor and so he is led to consider the importance of horizontal movement. But of what? Earthquakes in Yunnan and Kweichow are about 10 km deep so it seems that there is a gliding of the upper crust over the lower. North and South America have glided westward from Europe and Africa leaving the Atlantic exposed. In the Pacific it is the ocean floor which is moving westward and the inclined plane of earthquakes beneath Japan and the Kuriles is a manifestation of shearing between the old Pacific and the underside of Asia. Many other instances are given of meridional and latitudinal displacements of the Earth's crust, leading respectively to movement from higher to lower latitudes and to splitting of continents.

What is the driving force? Lee sees forces from the Earth's rotation as the only ones able to produce the two orthogonal types of motion. The resolved tangential component of centrifugal force is seen as the source of movement towards the equator, presumably (though this is not stated) in the form of *Polfluchtkraft*, the force on floating bodies that so occupied German geodynamicists in the 1920s. Westward movement is seen, on the other hand, as the result of variations in the rate of rotation of the Earth.

Very little of all this will move Western geophysicists, utterly committed to plate tectonics, to turn with enthusiasm to the Chinese literature. Lee's view of the world, so heavily dominated by what he saw in the field in China, is reminiscent of other qualitative syntheses that were made in the 1960s, all of which are now forgotten. And yet there is much to learn from this paper. Lee had to develop his ideas in the context of continental tectonics, a field which is still open to much discussion. The paper shows the fierce independence of the Chinese from the influence of the Russian school whose insistence on the dominance of vertical movement in the crust effectively kept the Soviet Union from participating in the excitement of the past ten years. It further shows that although Lee's views were by no means those of the West, there are grounds for discussion with Chinese earth scientists. Students brought up on Lee's interpretations would not find it difficult to understand, if not to accept, plate tectonics.

D. D.

Markers in heterogeneous nuclear RNA

from our
Molecular Genetics Correspondent

ONE difficulty in following the production of messenger RNA from its putative precursor, heterogeneous nuclear RNA (HnRNA), is the lack of markers which can be traced from the nuclear molecules to those in the cytoplasm. Such markers are needed both in the part of the HnRNA which is conserved and becomes the messenger and also in the part which is degraded. The importance of such sequences is emphasised by the use which they have been in tracing the path of production of ribosomal RNA from its large nucleolar precursor, where methyl groups identify the ribosomal RNA sequences and certain oligonucleotides have been found which may be part of the degraded sequences.

The marker usually taken to identify the mRNA sequence in HnRNA is the 200-long sequence of poly(A) which is added to the 3' end of the molecule before production of mRNA. The experiments which have shown that preventing addition of this poly(A) prevents production of mRNA suggest that it plays some part essential for messenger maturation. But these experiments have relied on drugs which block this process and in such cases it is always possible to argue that some unanticipated side effect of the drug is really responsible for its effect. The experiments now reported by Nakazoto, Edmonds and Kopp (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 200; 1974) therefore achieve two purposes. By identifying short oligonucleotide sequences of adenylic acid within HnRNA, they identify markers in it additional to the poly(U) sequences previously identified by Darnell and his colleagues; by comparing the metabolism of this oligo(A) with that of the 3' terminal poly(A), they confirm that the distinctive metabolism of the terminal poly(A) is indeed due to its position and not to some unexpected property of the drugs used to inhibit its addition.

Two drugs commonly used to follow the metabolism of HnRNA and mRNA are actinomycin and 3'deoxyadenosine (otherwise known as cordycepin). Low doses of actinomycin suppresses synthesis of rRNA but not HnRNA; higher doses inhibit both rRNA and HnRNA synthesis. Cordycepin does not inhibit synthesis of HnRNA, but by preventing the addition of poly(A) to the precursor, also seems to prevent production of mRNA, a strong line of evidence supporting the idea that HnRNA and mRNA have a precursor-product relationship. The oligo(A) recovered from within the HnRNA molecule

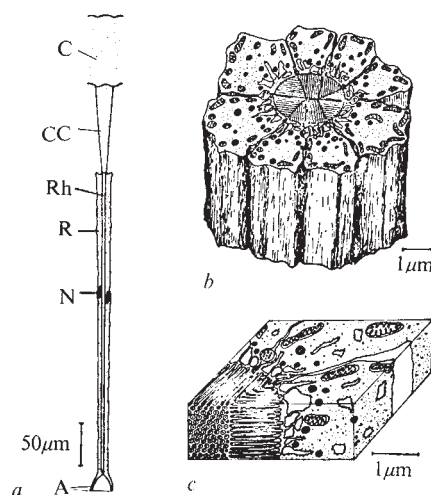
seems to be some 20-40 residues long, compared with the 200 bases of the terminal poly(A). Their metabolism is quite different. High levels of actinomycin which inhibit HnRNA synthesis prevent the synthesis of oligo(A); but have no effect on the addition to previously synthesised HnRNA of the terminal poly(A). Cordycepin has the opposite effect; it has no effect upon the synthesis of the internal oligo(A) but is effective in preventing addition of the terminal poly(A). By comparing these two sequences, these experiments provide strong support for the idea that the terminal poly(A) is indeed added by a mechanism quite distinct from that responsible for synthesis of HnRNA and that its addition is essential for the cleavage from HnRNA of mRNA and its transport to the cytoplasm.

The oligo(A) does not appear in cytoplasmic mRNA and so provides a marker for the sequences of HnRNA which are ultimately degraded in the nucleus. Because these experiments compared internal and terminal poly(A) sequences, they provide information only about the HnRNA molecules which contain terminal poly(A); Nakazoto *et al.* remark that this proportion represents some 15-40% of the total nuclear population. In the largest HnRNA molecules containing external poly(A), Nakazoto *et al.* calculate that there is on average less than one small internal sequence of oligo(A) per molecule. Although present in many of the HnRNA molecules, it is not therefore an inevitable feature. A more detailed examination of its occurrence and location will be necessary.

Miniaturised insect retina

from our Insect Physiology Correspondent

THE more closely the compound eyes of insects are studied, the greater becomes the respect which they inspire. Formerly regarded as primitive and inefficient structures, they are now revealing unsuspected complexities. Some of the latest acquisitions in this field are reviewed and new ones described by Snyder, Menzel and Laughlin (*J. comp. Physiol.*, **87**, 99; 1974) who deal with the functional specialisations of the 'fused rhabdoms' of the insect eye. In most of the photoreceptors of vertebrates and invertebrates the photosensitive pigment resides within the receptor cell in a column of higher refractive index than the surrounding medium. This column of photopigment acts as a dielectric waveguide which confines the light within the photoreceptor, where it is absorbed by the photopigment and transduced to receptor potential.



Structure of a typical cylindrical ommatidium with a fused rhabdom in the honey bee. *a*, General structure showing: C, corneal lens; CC, crystalline cone; R, retinula cells; N, nucleus; Rh, fused rhabdom; A, axons. *b*, Structure as seen with electron microscope: the fused rhabdom at the centre consists of sectors with parallel microvilli—the rhabdomeres of the eight retinula cells. *c*, A segment of the fused rhabdom showing the microvilli of two adjacent retinula cells. The microvilli of each rhabdomere are parallel in a hexagonal lattice.

Thus in vertebrate eyes, with few exceptions, each rod or cone is an independent waveguide, optically isolated from its neighbours. But in most compound eyes of insects several photoreceptors are fused together to form a centrally located common light guide, the rhabdom. In the worker honey bee (see figure), for example, eight photoreceptors (the retinula cells) each contribute a wedge of microvilli (termed a rhabdomere) and these eight wedges are fused together to form the common rhabdom. The parallel microvilli carry the photopigment aligned on the microvillous membrane so that each rhabdomere is sensitive to the plane of polarisation of the incident light. There is increasing evidence that the fused rhabdom is formed by rhabdomeres with different spectral absorptive powers, so that, given the necessary neural connections, spectral discrimination is possible in a single ommatidium. This will make possible a fine-grain colour vision, permitting hue discrimination in a very small field of vision.

But there are more subtle properties of the fused rhabdom which the authors have brought to light. The retinula cells of one cylindrical unit, or ommatidium, can interact both electrically and optically. Electrical coupling between cells occurs, and when this takes place between cells with microvilli aligned in different directions, it will impair the sensitivity to polarised light. On the other hand, if coupling occurs between cells with different photopigments, this

will result in a broadening of the spectral sensitivity curves of each and may be responsible for the double peaked spectral sensitivity curves that have been obtained from single retinula cells.

Optical coupling can take two forms. In some compound eyes, such as those of dragonflies and some Diptera, the rhabdoms are tiered and there is a direct filtering of light through successive photoreceptors with different properties. But even when the rhabdomeres are fused in a single column, since this acts as a single light guide, the absorptive properties of each rhabdomere will influence the light as it passes down the column. Indeed each rhabdomere acts as if it were an absorptive filter in front of all the others: the rhabdomeres function as lateral absorption filters. As a result the shape of the spectral sensitivity curve is more or less independent of the amount of light absorbed: the flattening of curves by self-absorption is prevented by optical coupling. This means that, as a consequence of optical coupling, each retinula cell can have a high absolute sensitivity while preserving its narrow spectral sensitivity curve.

In this way arthropod photoreceptors have solved the problem of collecting the small amount of light available to them and have increased the efficiency of quantum capture without loss of spectral sensitivity. Moreover, unlike electrical coupling, optical coupling within the ommatidium confers these benefits without loss of sensitivity to the plane of polarisation of the incident light. In many forms it looks as though polarisation sensitivity has been a by-product of rhabdom structure without much functional significance. Where a high level of polarisation sensitivity exists, it is found to be the result of a specialised arrangement of rhabdoms to give maximum sensitivity independent of spectral discrimination—as in the ninth retina cell of the honey bee, or the eighth cell in the fly.

In vertebrates the same problem of combining high absolute sensitivity with well defined spectral discrimination has been solved by having a dual system of rods and cones for low intensity and high intensity vision respectively. But it seems that certain deep sea fishes have also developed a system comparable with that of insects in which optical coupling provides a visual system which combines high spatial acuity, absolute sensitivity and colour discrimination in a compact array of photoreceptors behind a dioptric apparatus of extremely low aperture. So far as the insect is concerned, the ommatidium cannot be considered as a loose collection of receptors sharing the same dioptric apparatus; it must be seen as a highly integrated unit with remarkable discriminatory powers.

Carcinogenicity and mutagenicity

from a Correspondent

A WORKSHOP meeting convened by the International Agency for Research on Cancer and the Catholic University of Louvain was held in Brussels on December 10-12 to discuss approaches to assess the significance of experimental chemical carcinogenesis data for man. The meeting was chaired by Dr P. N. Magee (Middlesex Hospital Medical School, London) and the topics included comparative metabolism of chemical carcinogens, chemical carcinogenesis *in vitro* and mutagenicity tests.

Comparative metabolism

A. H. Conney (Hoffman-La Roche, Nutley) discussed the marked species differences in metabolism of foreign chemicals and their importance in determining the outcome of exposure of animals or man to chemical carcinogens. He emphasised the great difficulties and dangers involved in studying the metabolism of carcinogens in humans and the possible value of metabolic studies on non-carcinogenic chemicals. Recent work on metabolism of chemical carcinogens *in vitro* has included the use of biopsy or postmortem samples of human liver and preparations of human placenta, leukocytes or foreskin. E. Arrhenius (University of Stockholm) described how chemical carcinogens are metabolised, usually by microsomal enzyme systems which might activate or inactivate them. N-oxygenation was claimed to activate and C-oxygenation to inactivate aromatic amines, the reactions differing in their dependence on cytochrome P_{450} and in their response to inhibitors such as methylmercury compounds. The active metabolites, usually of electrophilic character, react with components of the cell nucleus and endoplasmic reticulum.

The evidence that N-nitrosamines are activated by enzymes to form alkylating agents whereas N-nitrosamides can form similar products in the body without enzymes was mentioned by R. Montesana (International Agency for Research on Cancer, Lyon). The organotropic action of the nitrosamines may be determined by the distribution of the activating enzymes but metabolism, although necessary, is not a sufficient condition for carcinogenesis. Human liver preparations metabolise dimethylnitrosamine *in vitro* at a comparable rate to rat liver and show a similar pattern of nucleic acid methylation. F. J. Wiebel (National Cancer Institute, Bethesda) described metabolic pathways of polycyclic hydrocarbons which lead to less or more carcinogenically-active products. Epoxides may be the active

metabolites with dihydrodiols, phenols and quinones as less active products. The enzymes concerned are present, and can be induced, in mouse skin and are inhibited by 7,8-benzoflavone which also inhibits skin carcinogenesis. Inhibitory effects of actinomycin D and cycloheximide suggest a role of messenger RNA in the induction of the enzyme. Differences in the activities of aryl hydrocarbon hydroxylase in human leukocytes may provide a basis for predicting susceptibility to these carcinogens in man.

P. L. Grover (Institute of Cancer Research, London) discussed the significance of polycyclic hydrocarbons in tobacco smoke for the induction of human lung cancer, suggesting the possible production of somatic mutations by enzymatically-formed epoxides. Direct evidence, including *in vitro* studies with human lung microsomes, and indirect evidence for formation of epoxides was presented and the importance of their further metabolism by hydrolases and glutathione conjugation was discussed. E. Huberman (Weizmann Institute, Rehovot) described a highly sensitive cell-mediated assay for mutagenesis in mammalian cells in which mutation to 8-azaguanine resistance was scored in V79 Chinese hamster cells, growing on layers of lethally irradiated cells which still retained the capacity to metabolise chemical carcinogens. Increased mutation rates, inhibited by 7,8-benzoflavone, were obtained with benzo[a]pyrene and methylcholanthrene. N-methyl-N'-nitro-N-nitrosoguanidine was active without the irradiated cell layer. Mutagenic activity correlated with carcinogenicity. P. T. Iype (Paterson Laboratories, Manchester) described methods for the *in vitro* culture of liver parenchymal cells with characteristic morphological, biochemical and immunological properties including contact inhibition, the presence of desmosomes, glycogen, ligandin, tyrosine aminotransferase and the capacity to produce serum albumin. Transformation was obtained with methylnitrosourea and other chemical carcinogens and confirmed by transplantation.

Testing for carcinogenesis

In vitro models for carcinogenesis testing were discussed by J. A. DiPaolo (National Cancer Institute, Bethesda) who emphasised quantitative experiments using mammalian cells and the production of cancer by transplantation of transformed cells into animals as the end point. Syrian hamster fibroblasts growing on irradiated rat cells were transformed by polycyclic hydrocarbons and the results correlate with carcinogenic potency *in vivo*. Toxicity reactions and transformation were separate and distinct processes. A similar banding pattern was observed in the chromo-

somes of transformed cells and tumour cells but these were considered secondary rather than causal. Pretreatment of the fibroblasts by irradiation or with methyl methanesulphonate enhanced the effect of some chemical carcinogens. Some compounds, including nitrosamines, were negative in the *in vitro* system but gave positive results if the embryos from which the fibroblasts were derived were exposed to the carcinogens transplacentally. The replica plating technique of Lederberg has been applied by T. Kuroki (University of Tokyo) to the selection of ultraviolet sensitive clones from mouse cell lines and of mutants induced in BHK-21 cells exposed to N-methyl-N'-nitro-N-nitrosoguanidine.

Testing for mutagenesis

Possible damage to the genetic material of man and animals from exposure to pesticides in the environment was pointed out by R. Fahrig (Zentral-laboratorium für Mutagenitätsprüfung, Freiburg), who described a range of tests for mutagenicity of such environmental chemicals with emphasis on the reasons for negative results. These included insensitivity of the system used, lack of activation of the compound and failure of penetration of the compound to the critical sites in the cells. Little is known about long-term exposure of animals to mutagens. The known liver microsomal activating systems for chemical mutagens were surveyed by N. Loprieno (Institute of Genetics, Pisa) whose group has used forward mutation and mitotic gene conversion and recombination with *Saccharomyces* as the test organism. The mutagenic activity of ethyl methane-sulphonate, a direct alkylating agent, is enhanced by mouse liver microsomes, but this effect was not explained. T. Sugimura (National Cancer Center Research Institute, Tokyo) summarised current work on AF2, a nitrofurant derivative used for about 7 years as a food additive in Japan. This compound gave positive results for DNA damage in repair tests and was positively mutagenic in *Escherichia coli* although negative in the *S. typhimurium* mutants of Ames. Carcinogenicity testing has so far proved negative and the compound was inactive in the sebaceous gland inhibition test in mouse skin. The great difficulties in extrapolating these results to man were discussed.

The positive correlation between the mutagenicity of selected chemical carcinogens in *Neurospora crassa* and their carcinogenicity in laboratory animals was emphasised by F. J. de Serres (National Institute of Environmental Health Sciences, Bethesda). He described a system of specific locus mutations in *Neurospora* which showed that chemical carcinogens produce

mainly base-pair substitution mutations, by contrast with the conclusions of some other workers that many potent carcinogens induce frame-shift mutations. He concluded that it may eventually be possible to develop a battery of tests that will permit the reliable prediction of carcinogenicity and mutagenicity. Finally, G. Röhrborn (University of Heidelberg) reviewed the mutation theory of cancer, emphasising that most human cancers do not show a definite mode of inheritance, though chromosomal aberrations are seen in human tumours (for example, Bloom's Syndrome) and carcinogens of various kinds interact with DNA. Rats treated with N-nitrosomorpholine or N-butyl nitrosourea showed evidence of chromosomal damage in the bone marrow and work is in progress to develop tests for carcinogenicity and mutagenicity in the same species of animal.

Superconductivity of metastable alloy films

from our Materials Science Correspondent

In the early fifties, Buckel and Hilsch in Göttingen deposited mixtures of metals such as bismuth and tin by evaporation on to substrates cooled by liquid helium, and so made super-saturated alloy films some of which showed superconducting behaviour to unexpectedly high temperatures. The method has been designated vapour quenching. This work has in recent years stimulated a fluctuating follow-up, first at IBM and now at Bell Telephone Laboratories, research which has gone in parallel with the growing amount of research on alloys cooled ultrarapidly from the melt. Both vapour-quenched and melt-quenched alloys are apt to be supersaturated, sometimes grossly so, and are thus in metastable form. Uncovenanted phases, not indicated by the phase diagram, are also apt to be formed.

The group at Bell Telephone have now published a detailed account of their recent researches on the superconductivity of vapour-deposited films (Testardi, Wernick, Royer, Bacon and Storm, *J. appl. Phys.*, **45**, 446; 1974). One novel feature here was that the substrate was not cold, but on the contrary held above room temperature during deposition. Instead of evaporation, the comparatively new method of getter-sputtering was used, in which the vapour species are generated by bombardment of metal targets by argon ions, in an atmosphere cleaned by gettering. The alloys, mostly high-melting compositions with superconducting major constituents, were found to produce metastable phases, as evidenced by X-ray diffraction. Com-

binations such as Hf/Nb, Mo/Nb/Re, Re/W, Rh/Zr were examined.

The superconducting transition temperatures, T_c , are plotted as a function of deposition (that is, substrate) temperature, and the generalisation emerges that T_c peaks for deposition temperatures which are close to phase-transformation temperatures in the equilibrium diagrams. The alloys studied mostly have equilibrium phases of δ , α -Mn or similar structural type near the temperatures which led to the highest values of T_c . The investigators conclude that structural instability enhances the superconducting transition; presumably, the phase which is in process of being formed is one which, in its normal state, is especially favourable for superconduction. But Testardi *et al.* were generally not able to identify with certainty what phases, metastable or otherwise, were in fact present in their films; they did establish that some X-ray lines at least corresponded to abnormal structures. This work shows that getter-sputtering is an effective technique for forming alloy phases with unusual properties, and that this virtue of the technique is by no means restricted to experiments with cold substrates.

At first sight, deposition of these alloys on a heated substrate is entirely distinct from deposition of bismuth or copper alloys on substrates held at 4 K. In fact, the difference is only apparent. Testardi's alloys are so refractory that even at temperatures around 400–600°C, diffusion in the bulk is very sluggish. Where the alloys have phase transitions in this temperature range, the new phases form very slowly when the alloy is cooled from the melt, whereas formation from the vapour permits the newly arriving, momentarily mobile atoms to form themselves readily into the equilibrium phase or a metastable transition phase of related structure. (It seems that the metastable structures form most readily in the immediate vicinity of a temperature at which a phase change would take place according to the phase diagram.) What Testardi and his colleagues have introduced is a form of vapour-quenching at elevated temperatures in which the film is transiently in a labile, adaptable state and is therefore at once frozen into instability.

Two of the alloys investigated, Mo₃Ru₃ and Ru₃W₃, also show exceptional hardness and corrosion resistance (Testardi *et al.*, *Met. Trans.*, **4**, 2195–2198; 1973) which might make such alloy films interesting for use as thin-film resistors, optical mirrors, razor blade coatings and so on. Since these alloys have now also been shown to have useful superconducting characteristics, they may also prove of value for use in superconducting memory devices.

Where does a membrane end?

from a Correspondent

THERE is currently a considerable amount of effort being expended in attempts to define the molecular structure of the surface membranes of cells. Unfortunately, there is no adequate definition of a 'membrane protein' and workers in this field compromise, either explicitly or, more often, implicitly, by adopting operational definitions. Singer and Nicholson (*Science*, **175**, 720; 1972) distinguish between 'integral' and 'peripheral' membrane proteins largely on the basis of their ease of solubilisation from membranes. This is, of necessity, an arbitrary decision: an integral membrane protein is one which remains attached to the fragmented vesicles which usually comprise 'purified membranes'. The problem with this approach is illustrated by the following comparison. A large fraction of the total cellular sialic acid is accessible to trypsin and therefore presumably is at the surface; however, membranes purified by some of the most commonly used procedures (Warren *et al.*, in *Specificity of Cell Surfaces*, edit. by Davis and Warren, 1967) contain only a small fraction of the total sialic acid. Does one conclude that the sialylated molecules are peripheral membrane proteins, or extracellular material, or alternatively, that the membrane preparation is inadequate? Clearly, it boils down to a semantic argument avoided only by operational definitions.

There is also the opposite problem of association, artefactual or otherwise, of non-membrane proteins, for example serum proteins, with cells and membrane preparations. One could propose, as a definition of a true membrane protein, that it be synthesised by the cell itself and possess a hydrophobic region inserted into the lipid bilayer. This definition also has a number of disadvantages; not least that it is, in practice, difficult to determine whether a particular protein is rooted in the lipid bilayer. It would also exclude proteins synthesised elsewhere and inserted into the membrane of a cell. There is clear precedent for such an export-import system in the case of egg yolk proteins which are synthesised in the liver.

Two recent papers illustrate the problem. Peterson, Rask and Lindblom (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 35; 1974) report that HL-A antigens released from 'crude spleen cell membranes' by papain digestion contain the protein β_2 -microglobulin, previously described as a protein present in serum and urine. Peterson *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **69**, 1697; 1972) had earlier found that this protein can be

detected immunologically on the surface of human leukocytes. Now they prove that β_2 -microglobulin is associated with several different HL-A antigenic specificities throughout papain treatment, a variety of column chromatographic procedures and immunoprecipitation by HL-A antisera. The β_2 -microglobulin was identified by electrophoretic mobility, antigenicity and tryptic peptide mapping. The HL-A specificities resided in polypeptides of about 33,000 daltons but, in each case studied, were associated with a polypeptide of 11,800 daltons indistinguishable from β_2 -microglobulin. The β_2 -microglobulin did not itself carry HL-A specificities. Does this association represent artefactual binding arising after digestion, or does it pre-exist on the intact cell and what is the origin of the β_2 -microglobulin? No evidence is presented as to whether the β_2 -microglobulin is synthesised by the spleen cells themselves. Previous workers, who have described a polypeptide of similar molecular weight in purified HL-A, found that it was synthesised by the cultured lymphocytes from which it was isolated (Cresswell, Turner and Strominger, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1603; 1973).

In an unrelated study Ruoslahti, Vaheri, Kuusela and Linder (*Biochim. biophys. Acta*, **322**, 352; 1973) discovered that an antigenic activity found on the surfaces of cultured chicken embryo fibroblasts is present in chicken serum. In this case the serum protein is of high molecular weight and migrates as an α -globulin. Antisera specific for this antigen were obtained by the unlikely procedure of treating intact fibroblasts with insolubilised papain and injecting the digest (after removal of the enzyme) into rabbits. Surprisingly, a monospecific serum was obtained. Using this serum, Ruoslahti *et al.* were able to show that the antigen is present on the surfaces of the fibroblasts (but not of several other cell types) and is synthesised by them (incorporation of 35 S-methionine). The fibroblasts from which the immunogen was obtained had been cultured in heterologous sera which do not themselves contain the antigen. Antigen was released into the medium of cells cultured in calf serum and its presence in chicken serum would suggest that it is also released from fibroblasts *in vivo*. The concentration of the antigen in chicken serum was estimated at several μ g per ml and is, therefore, much higher than the amounts of histocompatibility antigens and carcinoembryonic antigen which can also be detected in sera.

These two reports both show that one cannot always readily distinguish between membrane and serum proteins, let alone between 'true membrane proteins' and 'extracellular proteins'. In the

case of the fibroblast surface antigen the obvious interpretation is that the cells synthesise the antigen which, after a period at the cell surface, falls off into the serum. Whether the situation is the same or the reciprocal in the case of β_2 -microglobulin, it is clear that no firm line can be drawn which defines certain molecules as membrane proteins and others as non-cellular. The membrane rather than having an end, tails off into the medium. This is not a doctrine of despair for those hoping to define the molecular composition of cell membranes. Rather, it suggests the need for a more precise description of the molecular arrangement in temporal as well as topological and topographical terms.

Earth spin theory queried by doubting Thomas

by our Cosmology Correspondent

THE Ordinary Meeting of the Royal Astronomical Society held on March 8 was notably lively and lighthearted. The tone was set early in the proceedings, when the secretary, reading out the list of presents received encountered the item "De Witt and De Witt. 'Black Holes', presented by Mr Grey"; this provoked an appropriate audience response.

Even after the preliminaries, however, things did not immediately settle down into the technicalities of specialist papers; the first speaker, D. D. Thomas (Royal Greenwich Observatory), described "recent variations in the rotation period of the Earth", a topic which clearly aroused great interest among the majority of the fellows present, whatever their own specialist interests. The reason for presenting this item at this particular meeting was, Thomas said, the excessive press interest in the change in the length of the day (LOD) which occurred in December 1973.

As Thomas explained, this jump in the LOD was far from being an unusual event. A similar effect occurred in the preceding winter, and changes of the order of a millisecond in the LOD are not uncommon, although it is true that they occur more frequently in summer than in winter. And indeed the December 1973 event may not even have been very sudden, since the data show only that it occurred within a period of about 3 weeks. So why did it arouse such interest? Perhaps, Thomas suggested, the press were unusually sensitive to astronomical stories at that time, because of Comet Kohoutek.

Thomas then went on to discuss the variations in the LOD in more general terms. It is now generally accepted that seasonal changes in the rate at which

the Earth spins are caused by seasonal changes in the general circulation of the atmosphere, and he argued that meteorological effects could well be responsible for sudden changes in the LOD as well. He cast doubt on the reliability of evidence that earthquakes or sunspots might be causes of changes in the LOD. This is not to say that the overall trend in the acceleration of the Earth could not be influenced by solar activity, but "it is dangerous to argue a causal relationship between specific solar events and changes in the LOD".

Around the time of the recent Earth spin event, there were, it seems, changes in the pattern of westerly winds at the 500 mbar level, but Thomas declined to be drawn onto dangerous speculative ground, closing his talk with an emphatic statement that he would not attempt to say what causes the meteorological variations. As readers of the News and Views section will be aware, however, there is now compelling evidence that solar activity affects the very meteorological processes which seem to affect the LOD (see *Nature*, **246**, 384; 1973).

Earth plates as membranes

from our Geomagnetism Correspondent

In recent years several workers have sought to explain the origin of major tectonic activity away from plate boundaries in terms of hot spots, with or without associated mantle plumes. The best known example of the use of such an interpretation is probably the Hawaiian-Emperor island chain which is seen as the surface trace resulting from the motion of the Pacific plate over a stationary or near stationary magma source; but continental graben and rift valleys, such as the Rhine graben, have also been associated with hot spots. On the other hand, there are problems with this viewpoint and there are alternative, if no less contentious, explanations for both continental and oceanic mid-plate tectonic structures. The Rhine graben, for example, has an average width of 36 km and an estimated extension across the valley of 4.8 km; but it is not entirely clear how such features could result from hot spot or plume activity. And as far as the formation of the Hawaiian islands is concerned, the hypothesis that the chain derives from a propagating tensional fracture in the lithosphere which causes volcanic activity as it extends was put forward as early as 1942 and still receives much support today.

The chief difficulty with both the older and newer interpretations of the Hawaiian chain lies in substantiating them, although the former was recently

given a significant boost by Turcotte and Oxburgh (*Nature*, **244**, 337; 1973) who offered two possible origins for the required tensional stresses. The first of these involved thermal effects. Lithosphere created at an oceanic ridge by the cooling of mantle material cools further and increases in thickness as it moves away from the ridge. Such an elastic plate cooled non-uniformly will be subject to thermal stresses in the form of tensions which are parallel to the ridge, and thus aligned with the corresponding magnetic anomalies, but normal to fracture zones. Turcotte and Oxburgh were able not only to calculate the thermal stresses and show them to be sufficient to fracture the lithosphere but also to predict the direction of the tensional thermal stresses near Hawaii and show it to be in close agreement with a similar prediction using the theory for the plastic yielding and fracture of elastic solids.

The second type of tensional stress, the theory of which has now been developed at greater length by Turcotte (*Geophys. J.*, **36**, 33; 1974), is the membrane stress arising from non-spherical plate tectonics. The basic point here is that if the Earth were a perfect sphere plates would be able to move about without deformation but, because the Earth is in fact an oblate spheroid with an ellipticity of 0.00335, the lithosphere must deform when its latitude changes. A small plate at the equator, for example, will have two principal radii of curvature (6,378 km and 6,335 km), both of which will increase if the plate moves towards higher latitudes and both of which reach 6,400 km for a plate at either of the poles. Conversely, a small plate moving towards the equator will have its principal radii of curvature decreased. Normally such changes in the radii of curvature would produce bending stresses, but because the surface plates in this case are thin compared with the Earth's radius they will behave as thin shells in which the bending stresses may be neglected in comparison with the membrane stresses arising from stretching the plates to different radii of curvature.

In principle, therefore, it should be possible to determine the membrane stresses associated with the deformation of the Earth's moving plates using the already developed theory of thin shells—and this is what Turcotte has tried to do. For an unstressed thin shell whose radii of curvature are increased, the result will be a tension at the edge and a compression in the interior; for a decrease in the radii of curvature the situation will be reversed. But, first, do such membrane stresses actually exist in real plates? Plates move with velocities in the range 1-10 cm yr⁻¹, thus requiring about 10⁸ yr to undergo a significant change in latitude. It is usual

to regard the lithosphere as an elastic medium on a short time scale; but on the geological time scale will plastic yielding occur, relieving the membrane stresses? As Turcotte points out, at "modest" depths plastic yielding is to be expected because of the higher temperatures involved. But he also notes that the relief of membrane stresses requires plastic flow throughout the whole thickness of a plate and that there is abundant evidence (for example, from major fault systems) for the absence of long term plastic flow in near surface rocks.

Having disposed of this point (which is not the Aunt Sally it might at first seem), Turcotte goes on to develop the theory of membrane tectonics, generally along the lines mapped out by Novozhilov (*The theory of Thin Shells*, Noordhoff, 1959). For the real Earth the determination of the membrane stresses is, of course, an insolubly complex problem; surface plates have irregular shapes and each element of each large plate has its own radii of curvature which change in accordance with the particular variation in latitude. Turcotte thus adopts a highly simplified model in which the initially unstressed plate is represented by a circular segment of a spherical shell and in which the deformed plate is a similar spherical dome but with a slightly larger or smaller radius of curvature. In this scheme, deformation is produced by a radial surface force on the shell.

What emerges from this analysis is that a change in the latitude of the modelled lithosphere can result in membrane stresses of up to several kilobars. The stress required to produce a fracture in the real lithosphere is not precisely known, although stresses on active fault zones are known to be of the order of 0.1 kbar and laboratory work has shown the strength of mantle rocks to be of the order of 10 kbar. It may thus be fairly safely presumed that membrane stresses of several kilobars would indeed be sufficient to fracture the lithosphere, although thermal stresses and the stresses driving the plates may also play a part. Turcotte further shows that membrane stresses of about 2 kbar should lead to gravity anomalies of the order of 10 mgal, which is the magnitude of the anomalies actually observed.

Whether or not the importance of membrane stress is substantiated, Turcotte's analysis can only strengthen the hand of workers such as Jackson and Wright (*Petrology*, **11**, 405; 1970) who see the Hawaiian chain in terms of a propagating fracture rather than a hot spot. Moreover, membrane stresses seem to be the more capable of explaining the finite extension of rift valleys—as the extension needed to relieve the stresses.

West Atlantic abyssal circulation during the past 120,000 years

Detmar Schnitker

Department of Oceanography, University of Maine, Walpole, Maine 04573

Three faunal assemblages of benthic foraminifera are associated with the three abyssal water masses of the West Atlantic. Faunal distribution 15,000 yr ago indicates cessation of Arctic bottom water flow and a modified supply of Antarctic Water during the last full glacial period, resulting in an apparent warming of the bottom water. Faunal distribution and thus the deep sea circulation were similar to present conditions during the last interglacial 120,000 yr ago.

STUDIES of the remains of planktonic microorganisms from deep sea sediments reveal the climatic and oceanographic fluctuations that occurred in the surface waters of the world's oceans during the late Tertiary and particularly the Quaternary¹⁻⁶. Early studies of the abyssal benthonic foraminifera pointed to an apparent homogeneity of the deep-sea faunas⁷⁻⁹. Probably the past experience of micropaleontologists working within small areas and with very variable shallow water faunas made them insensitive to, or suspicious of, the small but real differentiations that can be found in deep-sea material.

Living (preserved) and total (empty shells) benthonic foraminiferal faunas were studied from West Atlantic sediment samples. Among the approximately 150 species of calcareous foraminifera of this area, three consistently recurring assemblages can be recognised whose areal distribution is shown in Fig. 1. Each of these assemblages is named after its most conspicuous member species and is closely associated with one of the three abyssal water masses that are present in the West Atlantic Ocean. The first, or *Epistominella exigua* fauna, occurs in its pure form in samples north of 45°N latitude only and is contained within the contours of the 1.9° C potential temperature of the Arctic bottom water, as shown by Worthington and Wright¹⁰. The second faunal assemblage, dominated by *Osangularia umbonifera*, is best defined within the 1.5° C potential temperature contour of the Antarctic bottom water, south of 30°N latitude. The transition from the *E. exigua* fauna to the *O. umbonifera* fauna is at approximately 35°N latitude, the area where the Arctic and Antarctic bottom water meet and lose their identity¹⁰. That indeed the water characteristics and not bathymetry control the faunal distribution is shown by the *Epistominella exigua* fauna which occurs at shallower depths in the northern portion of its range than further south, thus paralleling the descent of the Arctic bottom water from 500 m at the Denmark Straits to nearly 5,000 m north of Bermuda. Samples located on the continental rise and slope and on the mid-Atlantic ridge contain faunas in which several species of *Hoeglundina*, *Uvigerina* and *Gyroidina* are abundant. With a few exceptions these samples are contained between the 2° C and 4° C potential temperature contours. These faunal assemblages are associated with the lower North Atlantic deep water.

Disagreement between the postulated association of faunal assemblages and water masses appear to exist for four samples

collected within the Gibbs fracture zone, at the southern end of the Reykjanes Ridge. These samples contain typical *Epistominella exigua* assemblages, thus inferring the presence of Arctic bottom water at these sites. According to Worthington and Wright¹⁰ this area should be occupied by North Atlantic deep water. An easterly flow of bottom water through the fracture zone has recently been suggested^{11,12} and is supported here by faunal evidence. In a few areas, such as on portions of the Hatteras and Nares abyssal plains, the distribution of bottom faunas has been inferred only: carbonate solution at these great depths has modified the composition of the faunal assemblages and, in a few samples, nearly eliminated them altogether.

The information gained from the distribution of the present faunas can be applied to those of the past. Figure 2 shows again the distribution of three faunal assemblages for the latter part of the last glacial period. The core samples were taken from the upper part of the y-zone of Ericson *et al.*⁵, or the 17,000 yr BP level of the Climate/Long Range Investi-

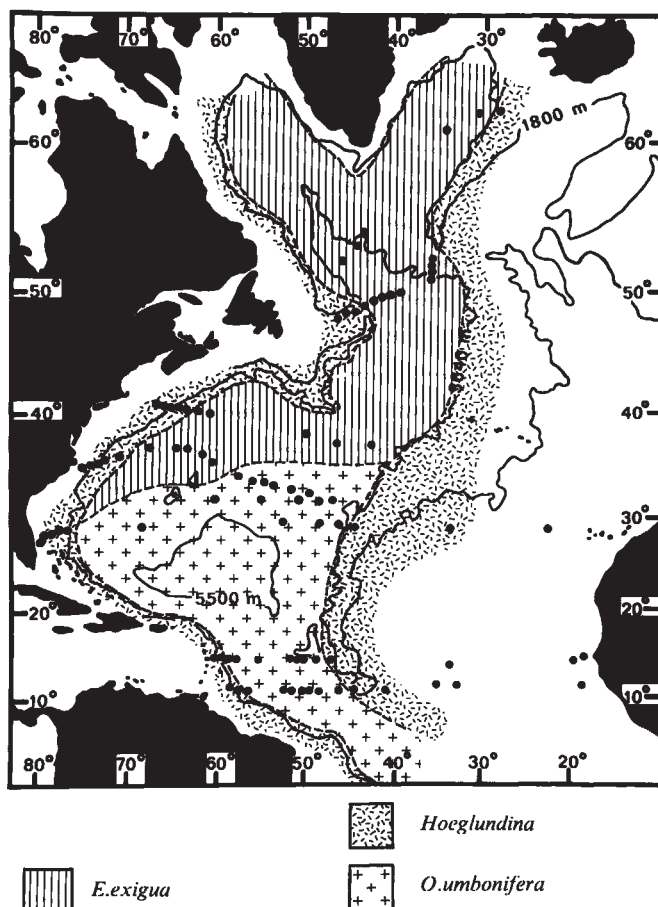


FIG. 1 Distribution of foraminiferal assemblages in the present western Atlantic Ocean. ●, Surface sample.

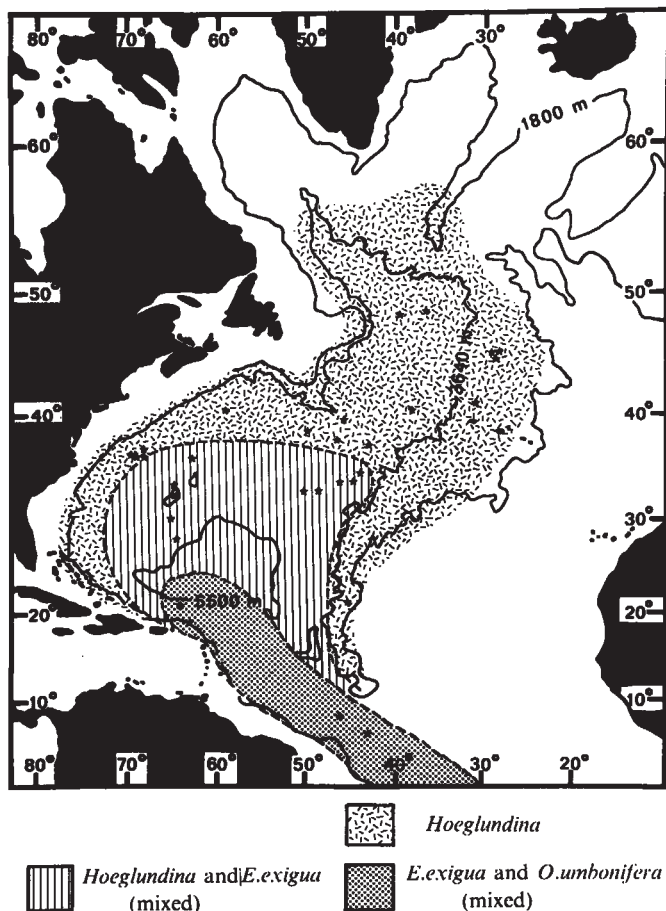


FIG. 2 Distribution of foraminiferal assemblages under glacial conditions, 17,000 yr ago. ★, Core location.

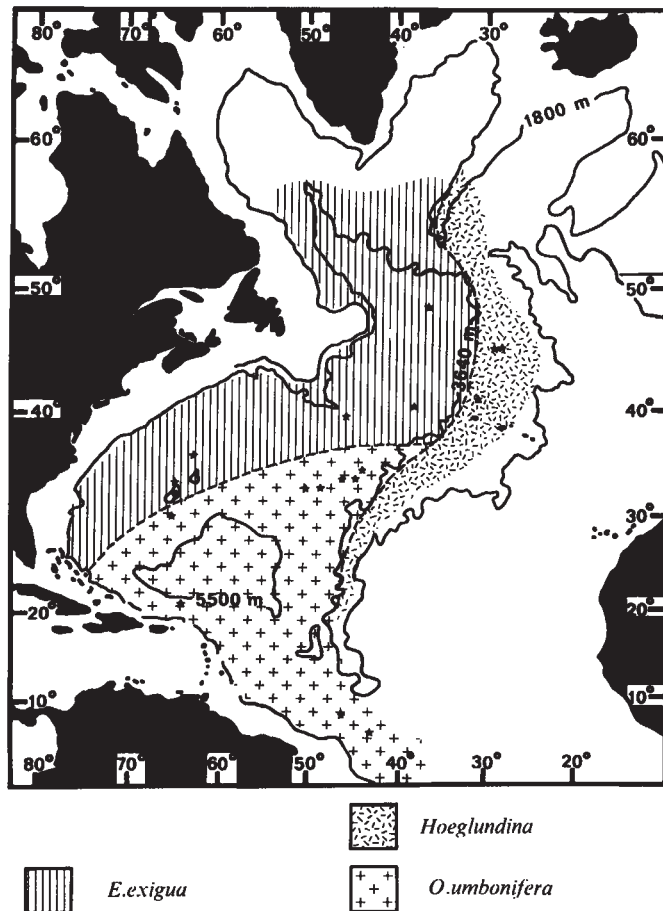


FIG. 3 Distribution of foraminiferal assemblages under interglacial conditions, 120,000 yr ago. ★, Core location.

gation Mapping and Predictions (CLIMAP) programme. Only one of the faunal assemblages of the present time existed during late glacial times, the *Hoeglundina* fauna, which at this time has invaded nearly all of the western Atlantic basin north of 40°N latitude. Towards the south, more and more elements of the *Epistominella exigua* fauna are represented so that south of 40°N latitude the bottom fauna gains a new identity as a *Hoeglundina-Epistominella exigua* mixed assemblage. Further south the faunal characteristics change again through a progressive increase of elements of the *Osangularia umbonifera* fauna and a concomitant decrease of the *Hoeglundina* fauna, so that south from about 22°N latitude the bottom fauna can be termed the *Epistominella exigua-Osangularia umbonifera* mixed assemblage.

Such a change in faunal composition and distribution was probably brought about by a change in the deep water circulation pattern and/or a change in the character of the water masses. Apparently, Arctic bottom water was not present and Antarctic bottom water, if it continued to enter the region, must have had characteristics different from those of modern Antarctic bottom water. A water mass with characteristics similar to those of modern Atlantic deep water occupied the entire northern portion of the basin, whereas a water mass which was probably produced by mixing of the northern and southern waters occupied the central portion of the basin.

Figure 3 shows the faunal distribution pattern for a time approximately 120,000 yr ago, towards the end of the last interglacial stage. The faunal assemblages and their distributions are nearly identical to those of today.

Such drastic changes of the deep thermohaline circulation between glacial and interglacial stages within the Atlantic Ocean have been proposed by Weyl¹²: reduced evaporation

in low latitudes led to the inflow of lower salinity water into the Norwegian Sea which then acquired a stable density stratification and a cover of sea-ice. Formation of Arctic bottom water ceased. A similar density stratification in the Antarctic would also have prevented the formation of Antarctic bottom water. A subpolar gyre was to form between the sea-ice front, south of Iceland and the subtropical

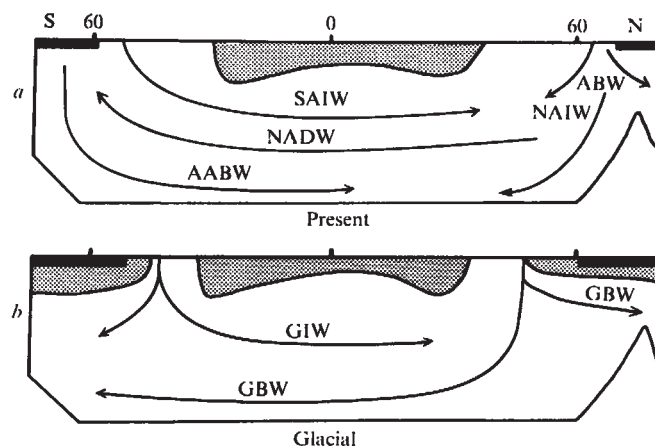


FIG. 4 Schematic N-S diagram of thermohaline circulation in West Atlantic Ocean under, a, interglacial and, b, glacial conditions. Modified from Weyl¹⁰. □, Low density surface water; ■, sea ice. SAIW, South Atlantic intermediate water; NADW, North Atlantic deep water; AABW, Antarctic bottom water; NAIW, North Atlantic intermediate water; ABW, Arctic bottom water; GIW, glacial intermediate water; GBW, glacial bottom water.

convergence, then at about 40° to 45°N latitude. Glacial bottom water which formed in this subpolar gyre, with temperatures similar to those of the present Atlantic deep water, was to be the only bottom water of the entire Atlantic Ocean. Figure 4 shows the generalised scheme of the Atlantic deep water circulation, modified from Weyl¹³, for glacial and interglacial conditions.

In the previous discussion specific qualities, such as temperature, salinity or dissolved oxygen content, have been purposely avoided and the general term 'water mass' used instead. Experience suggests that temperature may well be the most significant factor controlling the faunal composition and distribution. The present-day transition from the *Epistominella exigua* fauna to the *Osangularia umbonifera* fauna, however, takes place where both the Arctic and the Antarctic bottom water masses have reached a potential temperature of 1.9° C. The temperature in the transition area being equal for the two water masses, other factors, such as salinity or dissolved oxygen must be responsible for the faunal differentiation. The analytical techniques developed by Imbrie and Kipp⁶ may permit a quantitative assessment of the various environmental factors.

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(Duke University) and the RV *Gilliss* (University of Miami). Core samples were made available by the Bedford Institute of Oceanography and the Lamont-Doherty Geological Observatory of Columbia University.

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Nematocyte migration in *Hydra* influenced by tissue polarity

Rosalind L. Herlands & Hans R. Bode

Department of Developmental and Cell Biology, School of Biological Sciences, University of California at Irvine, Irvine, California 92664

In Hydra, migration of the desmoneme nematocytes takes place only in the direction of tissue polarity as defined by which end will regenerate a head.

THERE are many examples of cells that migrate as individuals through tissue or water to specific sites. Some of the influences involved in the apparently directed migration of these cells have been elucidated. Chemotaxis has been demonstrated in the aggregation of slime mould cells¹, in the movement of sperm toward the gonangium in the hydroid, *Campanularia*², and in the migration of leukocytes and lymphocytes to areas of infection^{3,4}. The orientation of the substratum upon which the cells migrate is known to orient the movements of cells *in vitro*⁵, and may well play a similar role in the organism. In other cases the movement may not be directed. Instead, cells migrate randomly until they reach the appropriate tissue where specific cell-cell contacts are made preventing any further movement. This mechanism has been postulated for the migration of primary mesenchyme cells in sea urchin embryogenesis⁶, and for the reconstitution of tissue layers from aggregates of cells^{7,8}. Here we describe an unusual type of influence which affects the migration of one type of nematocyte in *Hydra*, namely the polarity of the tissue. The mechanism underlying this influence may eventually prove to be one of those described above.

In *Hydra attenuata* four types of nematocytes (stinging cells) arise by differentiation from interstitial cells in the ectodermal layer along most of the body column^{9,10}. Each type is easily identifiable by the distinctive shape of its nematocyst. More than 80% of the mature nematocytes (H. R. B., and K. Flick, unpublished) migrate through the ectodermal layer¹¹ into the tentacles where they are mounted in pockets in the epithelial cells of the ectoderm. Desmoneme and atrichous isorhiza nematocytes are found only in the tentacles, whereas stenotele and holotrichous isorhiza nematocytes are mounted in the ectoderm throughout the body as well as in the tentacles.

Grafting and nematocyst migration

The large % of nematocytes in the tentacles suggests that when the nematocytes become mature they preferentially migrate in an apical direction. Vögeli¹² has shown that, indeed, small non-epithelial cells in the ectoderm do migrate preferentially in an apical direction. We have obtained more detailed evidence that supports this view. We grafted upper halves of *Hydra* (H123 in Wolpert's scheme¹³) labelled with ³H-thymidine to unlabelled lower halves (4B56F), and the reciprocal of labelled lower halves grafted to unlabelled upper halves. By periodically thereafter examining the unlabelled halves for labelled nematocytes, we found that nematocyte migration is strongly biased in an apical direction (R. L. H., and H. R. B., unpub-

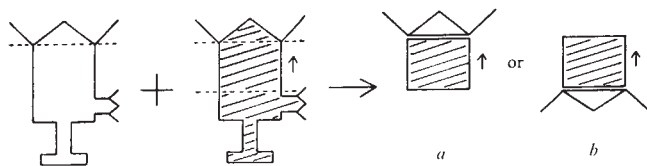


FIG. 1 Grafting procedure for analysing the migration of labelled nematocytes with or against tissue polarity. Dashed lines indicate sites of cutting. The pieces of tissue were removed and grafted together with the head at either *a*, the apical end of the gastric region or *b*, the basal end of the gastric region. Animals (cross-hatched) were labelled by injecting ^3H -proline into the gastric cavity one day before grafting.

lished). In fact, basal migration was found only if a bud was present, and then the nematocytes migrated into the bud. Migration into the peduncle is almost non-existent. These experiments indicate that the migration pattern of nematocytes is not simply one of random movement coupled with entrapment in the battery cells of the tentacles, but actually is one of preferential apical migration.

Once having reached the tentacles, nematocytes remain there and do not diffuse or migrate back into the body column. Campbell¹¹ has shown that by grafting a head containing nematocytes labelled with ^3H -thymidine to an unlabelled body that there is no measurable migration of labelled nematocytes into the body region. Further, large numbers of septate desmosomes between nematocytes in pockets of epithelial cells and the epithelial cells have been seen^{11,14} suggesting that once a nematocyte is mounted in a pocket it is permanently immobilised. Thereafter, the nematocyte remains on the tentacle until discharged or sloughed off with its host battery cell at the end of the tentacle. As the tentacles are in continuous growth¹¹ and the entire tentacle is renewed in about 6 d (H. R. B., and K. Flick, unpublished), the turnover of nematocytes is relatively rapid.

Chemotaxis or tissue polarity?

The apical directionality of migration may simply be due to a chemotactic attraction towards the head, or it may be due to some property of the tissue in the body column. The two possibilities can be distinguished by grafting a head to the basal end of a piece of the body column. In this case, if nematocytes are to migrate into the head, they must migrate in a basal, not the usual apical, direction. If nematocytes are guided by chemotaxis to the head, the position of the head at the apical or basal end should not affect rates of accumulation of nematocytes in the tentacles. If, however, some relatively stable property of the tissue influences the nematocytes to move selectively in an apical direction, one would expect to find much less accumulation of nematocytes in a basal head compared to an apical head. The following experiment was carried out to examine this.

Unlabelled heads of *H. attenuata* (hypostome and tentacles) were grafted to either the apical or the basal end of the gastric region of animals labelled with ^3H -proline (Fig. 1). In basal grafts, the host head was removed 3–5 h after grafting which inhibited regeneration of a head at the apical end during the course of the experiment. Wilby and Webster¹⁵ found similar results for basal grafts of *H. littoralis* while Wolpert *et al.*¹⁶, also using *H. attenuata*, left the host head on for 8 h to prevent regeneration. The nematocyst capsules were heavily labelled as was expected, since proline and hydroxyproline occur in large quantities in these structures¹⁷.

The accumulation rates of labelled desmonemes and stenoteles were measured as follows: periodically the heads

of five to eight grafts were removed and dissolved in 0.1 N NaOH plus 2.5% sodium dodecyl sulphate. The nematocysts, the only structures which survive this treatment, were analysed autoradiographically to determine the % of desmoneme and stenotele nematocysts that were labelled. The analysis was limited to these two types because they occur in greater numbers than do the two types of isorhizas and their distributions in the animal differ: desmonemes are mounted only in the tentacles whereas the stenoteles are mounted along the body column as well as in the tentacles. It should be noted that the time required for a mature nematocyte to migrate from the body column into the tentacles is short (less than 6 h), (R. L. H., and H. R. B., unpublished), and therefore, is not a factor in this analysis. The results (Fig. 2) indicate that the stenoteles accumulate at the same rate in the tentacles of a head grafted to the basal end of the gastric region as in one grafted to the apical end. In contrast, desmonemes accumulate in the tentacles of an apical head at a greater rate than in the tentacles of a basal head during the first three days. The difference between the rates of accumulation in apical and basal heads is significant (*t*-test, $P < 0.01$) on days 2 and 3, but not on any other day. Desmoneme migration, therefore, appears to be polarised. The fact that desmonemes accumulate at all in basally grafted heads, and the increasing migration rate into these heads after 3 d, might be interpreted, as the result of a gradual reversal of the direction of desmoneme migration under the influence of the basally grafted head.

Regeneration polarity

In this connection it is interesting to consider the effect of basally grafted heads on regeneration polarity in *Hydra*. The tissue of the body column is said to be polarised, as a piece of the column when removed will under normal circumstances always regenerate a head at the apical end and a foot at the basal end. Using *H. littoralis*, Wilby and Webster¹⁸ grafted heads to the basal ends of isolated gastric regions, removed the heads after a variable period, and examined the regeneration characteristics of the original gastric regions. They found that the pieces left in the graft combination for 1–2 d subsequently regenerated with the original polarity, that is, a head at the apical end and a foot at the basal end. After 4–5 d in the graft, the head regenerated at the basal end and a foot at the apical end. Thus, the basally grafted head reversed the regeneration polarity of the tissue. We repeated this experiment with *H. attenuata* (Table 1) and obtained similar results: regeneration with the original polarity after 1–2 d in the graft combination; intermediate forms such as bipolar animals

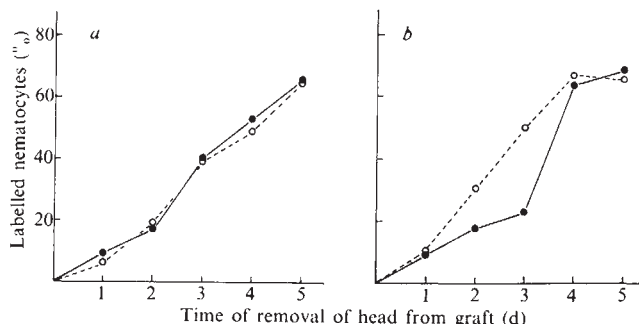


FIG. 2 Rate of accumulation of (a) stenoteles and (b) desmonemes in heads grafted to either the apical end (○) or the basal end (●) of a gastric region. The experiment was carried out twice. Each point represents the average value of 10–15 heads analysed individually. The difference between the rates of accumulation of desmonemes in apical and basal heads is significant (*t* test, $P < 0.01$) on days 2 and 3, but not on any other day.

TABLE 1 The effect of basal head grafts on host polarity

Time of proximal head removal (h)	No. of grafts	Original polarity	Type of regeneration		Reversed polarity
			Apical head and proximal tentacle	Bipolar	
24	10	7	1	2	0
48	10	5	1	3	1
72	10	2	2	5	1
96	7	2	0	1	4
120	7	0	0	1	6

For basal head grafts, the host head was removed 3–5 h after grafting a head to the basal end of the gastric region. As had been demonstrated earlier (R.L.H., and H.R.B., unpublished), removing the host head at this time instead of at the time of the basal head graft prevented normal head regeneration at the apical end. During the course of the experiments, which were carried out at $20 \pm 1^\circ\text{C}$, the animals were not fed, they did not bud, and no head regeneration at the apical end occurred while a head was attached to the basal end of the gastric region.

The four regenerating forms are defined as: original polarity, head regenerating at apical end; apical head and basal tentacle, head regenerates at the apical end and a single tentacle forms at the basal end; bipolar, heads regenerate at both ends; reversed polarity, a head regenerates at the basal end. The table represents the pooled data of two experiments.

(a head at both ends) after 2–3 d; and after 4–5 d in the graft, reversal of polarity. The intermediate forms were to be expected since the reversal of polarity is a gradual process: tissue near the basally grafted head is reversed sooner than the tissue near the original apical end or the gastric region as a whole (R. L. H., and H. R. B., unpublished). The kinetics of polarity reversal seem to parallel the kinetics of desmoneme migration into a basal head: slow at first, and rapidly approaching 100% after 4 d. Thus, the results are consistent with the idea that the direction of desmoneme migration is correlated with the regeneration polarity of the tissue.

A second experiment similar to the first provided more evidence supporting this hypothesis. In addition to grafting a head to the basal end, a peduncle was grafted to the apical end (Fig. 3). Wilby and Webster¹⁸ showed that this combination would reverse the regeneration polarity of the gastric region in 1–2 d instead of the 4–5 d necessary if only a head were grafted to the basal end. When we repeated this experiment with *H. attenuata* (Table 2), we also found rapid polarity changes: after 24 h, only one of 11 gastric regions regenerated according to its original polarity; polarity reversal was observed in more than half of the cases. The rates of nematocyte accumulation into apical and basal heads were measured as before. In this case (Fig. 4) there is no indication that the desmonemes migrate accord-

TABLE 2 The effect of basal head and apical peduncle grafts on host polarity

Time of head and peduncle removal (h)	No. of grafts	Original polarity	Type of regeneration			Reversed polarity
			Bipolar	Bipeduncle		
24	11	1	2	2		6
48	13	1	1	1		10
72	13	1	0	0		12
96	15	0	0	0		15

Both head and peduncle were grafted to the gastric region at the same time in both combinations. During the course of the experiments, which were carried out at $20 \pm 1^\circ\text{C}$, the animals were not fed and they did not bud.

The regenerating forms are the same as described in Table 1. Bipeduncle represents a peduncle which regenerates at both ends and in which no head is formed at all. The data is the sum of two experiments.

ing to the original regeneration polarity. If anything, the rate of accumulation of desmonemes is slightly greater in the basal than in the apical head, but the difference is not significant (*t* test, $P < 0.05$, except at 2 d). As before, the accumulation rate of stenoteles is the same for a head at either the basal or apical end of the piece of tissue.

Regeneration gradients

The correlation of regeneration polarity and direction of desmoneme migration which we have observed in two experiments suggests that desmonemes may be guided by the 'gradients' which are thought to control regeneration. Little is known about the nature of these gradients although several hypotheses have been put forward^{19–23}. In Wolpert's terms one could say that the desmonemes 'measure' the positional value of the cells they are moving over and migrate only in the direction of increasing positional value (with respect to head formation). During the reversal of polarity because of the basal head graft, the gradient of positional value is inverted, and the desmonemes continue to migrate 'up' the gradient, which is now towards the basal end of the gastric region. In this regard, our results also provide evidence that the changes which occur during the reversal of regeneration polarity of a gastric region occur throughout the piece, rather than being confined to the terminal cells which actually produce the new head and foot.

The mechanism underlying the active migration of desmonemes into the tentacles, or the 'measurement' of positional value, could be based on contact guidance, a restricted form of random movement (excluding basal migration), or some form of chemotaxis. The last is initially attractive because there is some evidence that diffusible substances play a role in the gradients of *Hydra*^{19,22} as well as in other systems such as the insect epidermis^{24,25} but it is doubtful that the desmonemes respond directly to these substances.

In *Hydra*, inhibition of head formation behaves as a gradient of substance diffusing from the head region. The gradient is very labile, vanishing within several hours after removal of the head²⁶. Also, grafting a head to the basal end of a piece of the gastric region establishes a new inhibition gradient, this time with the high point of the basal end, within 3–8 h (see above and refs 15 and 16). If the desmonemes were directly influenced by the gradient of inhibition, or gradient of positional signal¹⁹, one would expect them to migrate into basal tentacles in large numbers after 8 h, and not after 3 d as was observed. Because the properties of the inhibition gradient are not consistent with the observed desmonemes migration behaviour, the desmonemes probably do not 'read' this gradient.

Another possibility is that the desmonemes may respond to a substance diffusing from a graded distribution of sources along the length of the body column, whose density is highest at the apical end²². However, the rapid change (24 h) in tissue polarity in the second graft combination that we and Wilby and Webster¹⁸ observed would require an equally rapid reversal in the graded distribution of sources, or cell

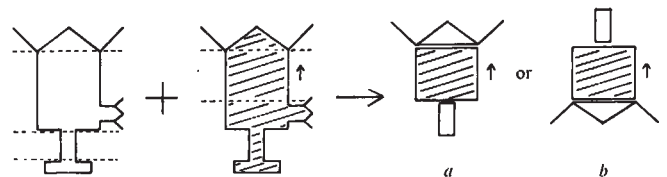


FIG. 3 Grafting procedure for analysing the effect of the peduncle on accumulation rates of nematocytes in basal or apical heads. Dashed lines indicate sites of cutting. The head and peduncle were grafted to the gastric region either *a*, in the original orientation or *b*, in the inverted orientation. Animals (cross-hatched) were labelled by injecting ^3H -proline into the gastric cavity.

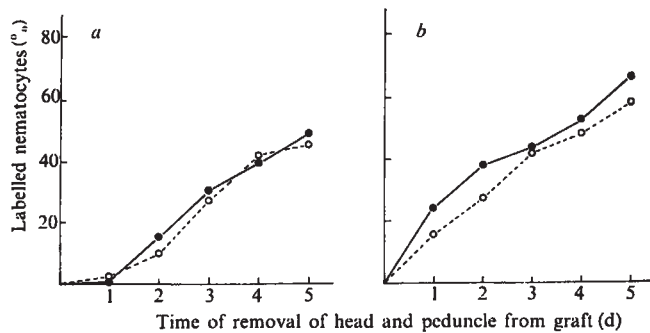


FIG. 4 Rate of accumulation of stenoteles (a) and desmonemes (b) in heads grafted to either the apical end (O) or the basal end (●) of a gastric region in the presence of a peduncle grafted to the opposite end. The experiment was carried out twice and each point represents the average value of 10–15 heads analysed individually. For the desmonemes, the differences between control and experimental points are not significant at the $P = 0.05$ level, but the second day point is significant at the $P = 0.02$ level.

type, which seems unlikely. This evidence would also appear to render untenable any theory in which tissue polarity is due exclusively to the graded distribution of a particular cell type.

Stenotele migration

In contrast to desmoneme migration, stenotele migration is not affected by the polarity of the tissue. Stenoteles will migrate into basally-grafted heads as rapidly as they will into apical heads. This is not simply the result of random movement coupled with entrapment in the tentacles for, as mentioned earlier, in normal animals stenoteles migrate almost exclusively in an apical direction. Only if a bud is present will stenoteles migrate basally (R. L. H., and H. R. B., unpublished), as stenoteles always migrate toward a head region, some form of chemotactic attraction to the head is probably involved.

Thus, desmoneme and stenotele migration are, at least in part, affected by different influences. Though other hypotheses can be constructed to explain the rates of ac-

cumulation of desmonemes in apical and basal heads, these hypotheses must take into account the rather different rates of accumulation of stenoteles and desmonemes in the basal heads in the first experiment. This renders unlikely those hypotheses based on a systematic difference in the experimental design, for such a difference would be expected to affect both nematocyte types in the same way.

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Super-relativistic phase velocities of radio source components

R. H. Sanders

National Radio Astronomy Observatory, Green Bank, West Virginia

A sudden injection of relativistic electrons at the centre of an extended dipolar magnetic field could lead to an apparent faster-than-light expansion of two synchrotron emitting regions. Subsequent injections of electrons would produce components which move outwards along the same axis.

INTERFEROMETRIC radio observations with transcontinental baselines reveal rapid fluctuations in the fine structure of some extragalactic radio sources,^{1,2} and in at least three cases these fluctuations imply expansion of small radio components at

velocities apparently greater than that of light, if these sources are at the distances indicated by the cosmological interpretation of their redshifts. In the case of the QSOs 3C279 and 3C273, the apparent separation velocities of double components are $6c$ and $4c$ respectively.² For 3C120, which has been variously designated as a Seyfert or N-type galaxy, the apparent separation velocity is $3c$ (ref. 3). BL Lac has also exhibited qualitatively similar rapid changes in structure. More recent observations indicate that the actual structure might be more complicated than the original simple models but the problem of explaining apparent faster-than-light motions of source components persists.

Classes of models

One might expect apparent faster-than-light expansion motions of relativistically separating components, if the direction of the motion is almost along the observer's line of sight⁵, but such models require somewhat contrived conditions⁶. Moreover, accumulated observations seem to reveal that these apparent faster than light motions are a not uncommon property of compact radio sources, whereas expulsion of components within 10° of the observer's line of sight is improbable.

A second possible explanation of this phenomenon is the "Christmas tree" model in which the radio source is assumed to consist of a number of independently blinking components spread over an extended region⁷.

There are three possible difficulties with this model. First, the luminosities of the blinking components must change such that limitations on the total flux variations from the source are not violated. Second, there should be just as many apparent super-relativistic contractions as expansions in radio sources. Third, in a truly random distribution of blinking components the position angle of the line connecting the two dominant components should also be a function that varies with time; in at least two cases, 3C279 and BL Lac, this position angle seems to be constant^{2,4}. Dent⁸ has answered this objection by suggesting that these random events are occurring in a disc seen edge on but this is improbable if the constancy of position angle is a general property of these sources.

A third possible class of models for sources with rapidly variable structure involves phase velocities of components^{1,9}. The specific model suggested here is of this class.

Particle injection in a strong dipolar field

I assume that the magnetic field of such a source has a dipolar form. The energy and relativistic particle source of the quasar is at the centre of the dipole, and far from this central object¹⁰ the magnetic field lines satisfy the polar coordinate equation

$$r = R_0 \sin^2 \theta \quad (1)$$

Variables are defined as shown in Fig. 1. For simplicity I assume that the observer's line of sight is perpendicular to the axis of the dipole.

At time $t_0 = 0$ a burst of relativistic electrons is emitted by the central object, and I assert that the total energy of these electrons is less than the total magnetic field energy of the dipole. So a train of electrons, the length of which is determined by the duration of the burst, will move out along every field line. The pitch angles of these electrons will be small for two reasons. First, the magnetic field strength drops off rapidly ($\sim r^{-3}$), and the quantity $P_{\perp}^2/B$ (where $P_{\perp}$ is the momentum of the electrons perpendicular to the magnetic field, B) is an adiabatic invariant of the electron motion. Second, electrons with high pitch angle will lose energy rapidly by synchrotron radiation in the central region of high field. Therefore, as the train of electrons travels out along the field lines, their synchrotron radiation will be confined to a narrow cone along the field line, and will not be directed toward the observer. At some time t_1 , however, when the train has reached r_1' (Fig. 1), the 'search light' of the electron train will sweep past the observer, who will see a synchrotron spot at a projected distance d_1 from the central object when the radiation reaches him. Since the dipolar field is symmetric about the equatorial plane, the observer will see two such spots separated by a projected distance of $2d_1$.

At a later time, t_2 , this train of electrons will have moved over the top of the field line and, still emitting synchrotron radiation forwards, will no longer be visible. But a similar train traveling along a higher field line will have reached r_2' at a projected distance of d_2 from the central object. The

two synchrotron spots will now have increased their separation to $2d_2 > 2d_1$. Thus, the radio source will seem to have two components separating with an apparent velocity of

$$V_s = 2(\Delta d)/\Delta t \quad (2)$$

where $\Delta d = d_2 - d_1$. Δt is not the difference in travel times of the two electron trains ($t_2 - t_1$), but the difference in arrival times of the synchrotron radiation from r_2' and r_1' at the observer. Radiation coming from r_2' has less far to travel to reach the observer (by a distance x), so

$$\Delta t = t_2 - t_1 - x/c \quad (2a)$$

where $x = \Delta d \tan \theta_0$.

This apparent separation velocity (equation (2)) does not represent the motion of two objects; it is a phase velocity of the emission region and is not physically limited by the velocity of light.

For a perfect dipolar field, equation (2) can be solved explicitly to obtain a numerical value for the apparent separation velocity of the synchrotron spots: $V_s = 4.4c$.

This model does not depend on the observer being in the equatorial plane of the dipolar field. In general, one will always observe two separating synchrotron spots regardless of orientation unless the line of sight is coincident with the axis of the dipole, in which case the observer will see an expanding ring. With impulsive injection of electrons, the apparent expansion velocities for orientations other than that described above will be less than $4c$ but greater than $2c$. Further, it is interesting that if particle injection were continuous instead of impulsive, one could see continuous synchrotron radiation along a line corresponding to the projection of the magnetic dipole axis on the plane of the sky—a jet.

Individual source parameters

Several conditions enable estimation of the physical parameters of individual sources. If: (a) the magnetic field is dipolar in form; (b) the energy density of the field is higher than that of the particles; (c) the relativistic particle energy density must be sufficiently great to explain the total radio luminosity up to a frequency of 50 GHz or so; and (d) the pitch angles of the radiating electrons must be less than 10° in the emitting regions; then with the measured separation of the double components in a particular source we may estimate the size (a) of the region over which particle acceleration takes place (that is, the radius of the equivalent current loop producing the dipolar magnetic field), the magnetic field strength in this region (B_0) and the total magnetic field energy (E_H). Assuming cosmological distances (Hubble's constant = $75 \text{ km s}^{-1} \text{ mpc}^{-1}$; $q = 1$) I find for 3C120 (ref. 3) $a = 0.3 \text{ pc}$, $B_0 = 1.0 \text{ gauss}$; $E_H = 3 \times 10^{53}$

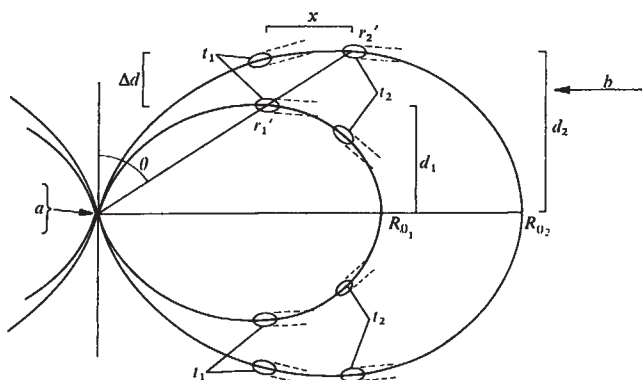


FIG. 1. Dipolar field geometry of a rapid variable structure radio source. a , Particle burst injected at $t_0 = 0$; b , line of sight.

erg; and for 3C279 (ref. 1) $a = 5$ pc, $B_0 = 0.3$ gauss; $E_R = 3 \times 10^{56}$ erg. The derived field energies are reasonable compared with total energies of fields and particles in quasars and radio galaxies ($\sim 10^{60}$ erg).

For both sources acceleration of electrons takes place over a large region, and it is unlikely that this acceleration occurs simultaneously throughout that region. The maximum separation velocity resulting from impulsive injection of electrons at the centre of a magnetic dipolar field is $4.4c$. But for these two sources the injection cannot be truly impulsive. This offers the possibility of producing arbitrarily high separation velocities of double components. Suppose, for example, that acceleration of particles first occurs near the centre of the equivalent current loop of the extended dipolar field. Trains of electrons moving up the higher field lines of the extended field (Fig. 1) would have a head start on electrons moving up lower field lines. The difference in arrival times of the two electron trains ($t_2 - t_1$) would therefore be decreased and a higher apparent expansion velocity would be observed. Thus the apparent component separation velocity of $6c$ in 3C279 could occur if the injection of electrons at the centre of the extended dipolar field is not impulsive.

Observational predictions

It is difficult to imagine how the orientation of the dipole could change on a time scale shorter than that between successive flares. So I expect that all motions of radio source components should be along the same axis—the axis of the dipole projected onto the plane of the sky. In all subsequent outbursts the apparent motion of the components should be along an axis having the same position angle as the first observed event. This seems to be the case for BL Lac (ref. 4).

Second, since the distribution of electron pitch angles in the synchrotron emission regions (spots) is strongly peaked around zero, I expect a resultant circular polarisation of the

radio emission from the individual source components of the order of a few per cent. The direction of the magnetic field is predominantly toward the observer in one of the spots and away from the direction of the observer in second spot. So the sense of the circular polarisation should be opposite in the two components of the double source. In other words, the relative intensity of the two source components should be different by a few per cent when observed in opposite senses of circular polarisation.

Finally, since the burst of electrons from the central object could be an extended event in time, some sources might have the structure of a double jet with a rapid outward-moving brightening at the two opposite ends of the jets.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Intermittent momentum transport in a geophysical boundary layer

THE development of flow visualisation techniques^{1,2} has prompted a renewal of interest in investigating the dynamics of turbulent boundary layers. The discovery that turbulence is generated intermittently, in "bursts"³, near the wall, is of particular significance. It has also been found that the ejections and intrushes of fluid that accompany the bursts of turbulence generation are dominant contributors to the Reynolds stress⁴⁻⁶. These ejections and intrushes extend well into the flow. It follows that momentum transport in turbulent boundary layers is an intermittent process and that the intermittency is not confined to the region near the wall where most of the turbulence production takes place.

Can such laboratory results on turbulence structure be applied directly to boundary layers of geophysical scale? The potential importance of intermittence-related phenomena in large scale flows has been discussed by Mollo-Christensen⁷

and by Grass⁴. The work by Dorman and Mollo-Christensen⁸ also gives some direct evidence for intermittent momentum exchange in the atmospheric boundary layer.

For some time, a colleague and I have been investigating the turbulent structure of an estuarine, tidal flow and interpreting our results in terms of contemporary research on boundary layers⁹. The continuing analysis of our measurements has included a search for evidence of intermittency in this boundary layer of geophysical dimensions. We have found that the turbulent transport of momentum, or the Reynolds stress, is highly intermittent and that the observations are consistent with the results of wind tunnel and laboratory measurements made at lower Reynolds numbers.

The field experiments were carried out in the Choptank River estuary on Chesapeake Bay. Vertical and horizontal current velocities were sampled every 2 s for approximately two tidal cycles. The overall depth at the measuring station was 8–9 m and currents were recorded at various distances above bottom using a pivoted-vane current meter¹⁰. The maximum tidal flow was approximately 75 cm s^{-1} yielding Reynolds numbers based on depth of the order of 10^6 – 10^7 during most of the cycle. At these flow rates the sampling interval of 2 s allowed detection of turbulence structure with horizontal dimensions ~ 1 m. Turbulent fluctuations in the horizontal current in the direction of the mean flow (u')

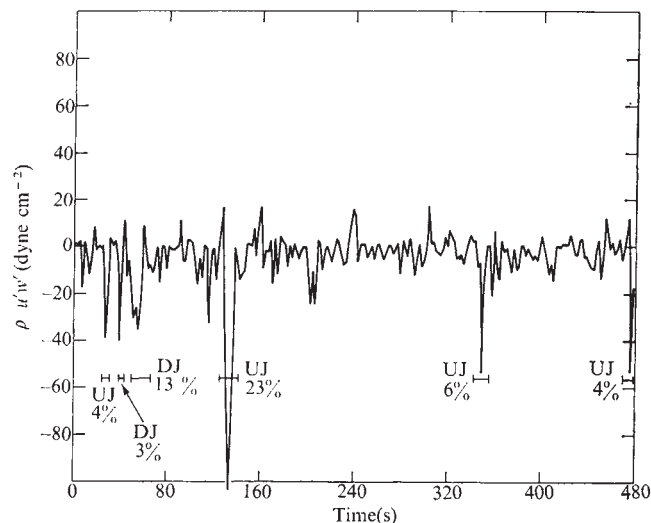


FIG. 1 Time series of the correlation between horizontal (u') and vertical (w') current velocity fluctuations at 2.25 m above bottom showing intermittent, large contributions to the Reynolds stress. Decelerating flood tide (current 62 cm s⁻¹). Run 541-570. $\rho u'w'$ (8 min average) = 5.16. Six events in 7% of the time account for 54% of the measurement of Reynolds stress.

and in the vertical current (w') were obtained by calculating deviations from a third degree polynomial fit to the sampled currents. The velocity fluctuations (u' and w') were then

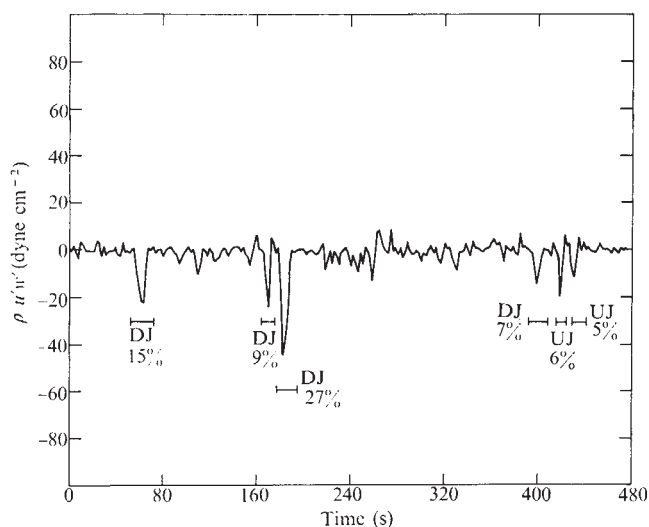


FIG. 2 Time series of the correlation between horizontal (u') and vertical (w') current velocity fluctuations at 1 m above bottom showing intermittent, large contributions to the Reynolds stress. Decelerating ebb tide (current 47 cm s⁻¹). Run 661-690A. $\rho u'w'$ (8 min average) = 1.98. Six events in 9% of the time account for 70% of the measurement of Reynolds stress.

correlated to yield the Reynolds stress component that defines the vertical transport of horizontal momentum, $\tau_{xz} = -\rho u'w'$, where ρ is the density of water. The averaging interval indicated by the bar over $u'w'$ was 8 min. The results presented here represent initial processing of data from 100 such intervals of 8 min taken during different phases of the tide.

Figures 1 and 2 show two typical intervals of 8 min in which individual values of $\rho u'w'$ are plotted against time. The Reynolds stress value is obtained from the average of these velocity correlations. Corrsin¹¹ has cautioned against referring to the individual products $\rho u'w'$ as the "instantaneous

Reynolds stress" but it is clear that there is intermittent structure in these 'events' which, when averaged, make up the Reynolds stress. Much of the final value of τ_{xz} is contributed by a relatively few, intermittent, highly correlated fluid motions. The UJs and DJs classify the kinds of events that transport the momentum—UJ signifies an upward jet of slower moving water ejected away from the bottom boundary into the main stream; DJ indicates a downward jet of faster moving water approaching the bottom. The percentage contributions of each of these events to the Reynolds stress value are shown. Table 1 summarises the relative contributions of events in different quadrants of the $u'w'$ plane to the Reynolds stress. These results are in good agreement with the laboratory data of Wallace, Eckelmann and Brodkey⁶, which were obtained from hot film measurements in an oil channel.

The frequency of momentum transporting events rather arbitrarily depends on the criteria used to define an event. Events in Figs 1 and 2 were simply taken as the six largest values of the products $u'w'$. If any value of $u'w'$ more than two standard deviations from the median is considered to be an event, the data treated so far indicate that events typically occur about every 25 s. This result may be compared with the wind tunnel data of Rao *et al.*¹². Their studies of the "bursting rate" as a function of R_θ (Reynolds number based on the momentum thickness θ) indicate that the period between events scales on the flow parameters of the outer part of the boundary layer, U and δ^* . (U is the mean flow velocity and δ^* is the displacement thickness.) Using the conclusions of Rao *et al.*¹² and typical values of U and δ^* from our data, the predicted period between events is about 35 s. The agreement of the observed and calculated frequency is close enough to indicate that we are dealing with the same kind of intermittent phenomena in the geophysical boundary layer.

Figure 3 consolidates data from 100 samples of 8 min of the type shown in Figs 1 and 2. The abscissa represents the percentage of the time occupied by individual products $u'w'$, selected on the basis of magnitude only, without regard to sign. The ordinate is the percentage contribution that these selected events make to the measurements of Reynolds stress. About 15% of the Reynolds stress is due to events occupying approximately 1% of the time, whereas some 60% of the stress is produced in only 10% of the time. If extrapolated slightly, this result is in good agreement with the wind tunnel observations of Willmarth and Lu⁵ who found that 99% of the stress value was contributed in 55% of the time.

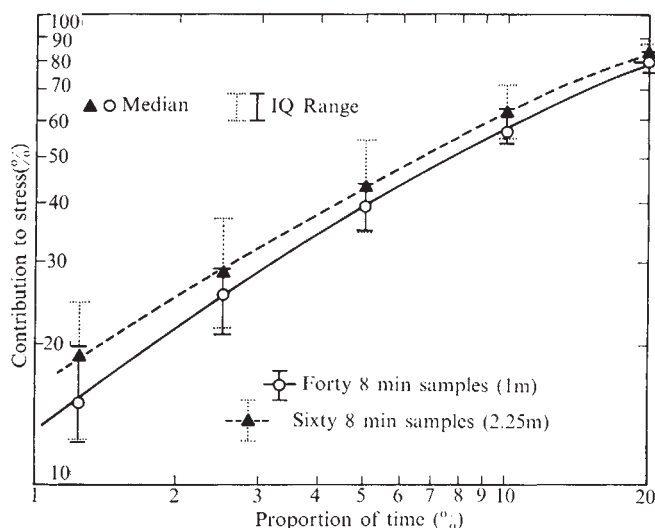


FIG. 3 The degree to which intermittent, momentum transporting events contribute a disproportionate share to the Reynolds stress. IQ, Interquartile.

TABLE 1 Relative contributions of events in the $u'w'$ plane to Reynolds stress (%)

Distance off bottom	$u' < 0, w' > 0$	$u' > 0, w' < 0$	$u' < 0, w' < 0$	$u' > 0, w' > 0$
1 m	65 ± 3	67 ± 6	-15 ± 4	-15 ± 4
2.25 m	73 ± 8	65 ± 8	-17 ± 6	-20 ± 8
Wallace <i>et al.</i> ⁶	~ 68	~ 60	~ -15	~ -15

Our measurements in an estuarine tidal flow indicate that the intermittent phenomena observed in wind tunnel and laboratory boundary layers can be scaled up to dimensions of geophysical interest. It may well be that the intermittence of momentum transport is a prominent and characteristic feature of most geophysical boundary layers.

CLIFFORD M. GORDON

Ocean Sciences Division,
Naval Research Laboratory,
Washington, DC 20375

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"Bursting" phenomena in the sea

INTEREST in the structure of turbulent boundary layers and in particular mechanisms responsible for the transfer of turbulent energy and generation of Reynolds stress has recently been stimulated by a series of laboratory flow visualisation experiments¹⁻⁵. These have indicated that Reynolds stress production is intermittent and associated with a sequence of motions known collectively as "bursting"³. Experimental evidence presented here suggests that similar motions can be readily identified in a geophysical boundary layer at length and time scales unattainable in the laboratory.

The observations reported here form part of a larger programme carried out between 1971 and 1973, in which systematic measurements of near bottom turbulence have been made in the Irish Sea. The primary objective of this study has been to determine by conventional means⁶⁻⁹ the frictional interaction between tidal currents and the sea bed and to extend previous observation^{8,9} of the turbulent structure of bottom boundary layers from estuarine and coastal waters to conditions more typical of circulations in the sea.

Measurements have been carried out at locations shown in Fig. 1, at which depths have varied between 10 and 60 m and maximum surface currents have been of the order of 1 ms^{-1} . The horizontal and vertical turbulent velocity fluctuations u and w have been measured at various heights in the bottom 2 m of the boundary layer using electromagnetic current meters¹⁰. These were mounted on a probe which was lowered to the seabed from the surface. Voltage analogues

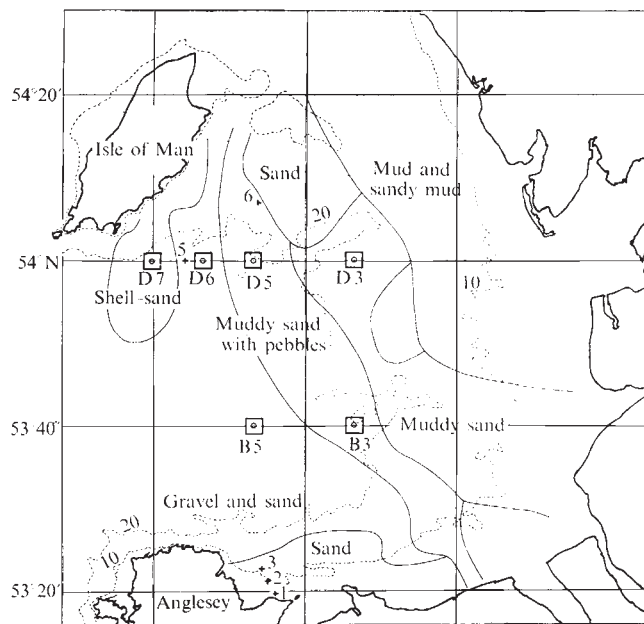


FIG. 1 Locations at which observations of bottom turbulence have been made in the Irish Sea between 1971 and 1973.

of the turbulent velocity components were transmitted to a ship at the surface by cable, recorded on magnetic tape and digitally on punched paper tape. It has been possible to measure simultaneously the contributions to the Reynolds stress ($-\rho u'w'$ where ρ is the fluid density) at two heights above the seabed over a wide range of flow conditions, depth and bottom sediments. Approximately 150 time series (10 min each) of u and w have been obtained, together with measurements of the velocity profile over the entire depth. Records have been replayed through low pass analogue filters into a data logger and digitised at intervals between 0.63 s and 0.08 s. The mean, standard deviation, skewness and kurtosis for each record were calculated before spectral analysis using standard digital methods. The turbulent spectra of u and w and the Reynolds stress cospectrum, corrected for the frequency response of the current meters and filters, non-simultaneous sampling and sensor misalignment, have been computed and are being used to determine those scales of motion contributing to the Reynolds stress.

Reports of bursting phenomena in laboratory experiments¹⁻⁵ have prompted us to examine our records for similar mechanisms. Figures 2 and 3, showing reconstructed analogues of u , w and uw from two different locations, indicate the intermittent nature of the stress input to the boundary layer by a pronounced asymmetry of the uw trace. Record 2/14, made with a sensor of diameter 10 cm and a time constant of approximately 1.0 s, has fewer high frequency components than record 11/4/2, obtained with a sensor of diameter 5 cm having an overall time constant of 0.1 s.

Inspection of the signs and magnitudes of u and w in Fig. 2 identifies the large values in the uw record as belonging to the bursting process and following the usual convention⁵, these events can be classified as follows:

- 1) $u < 0, w > 0, uw < 0$; an ejection of fluid away from the boundary.
- 2) $u > 0, w < 0, uw < 0$; a sweep or inrush of fluid toward the boundary.
- 3) $u > 0, w > 0, uw > 0$; a weak outward interaction of fluid from the boundary.
- 4) $u < 0, w < 0, uw > 0$; a weak inward interaction of fluid toward the boundary.

In particular those terms contributing to the ejection and

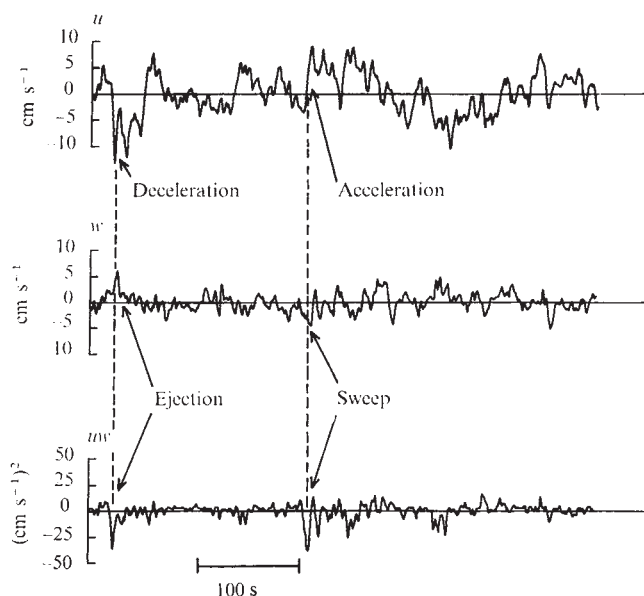


FIG. 2 Bottom turbulence record 2/14, station 2, depth of water 15 m. Analogues of u , w and uw at a height of 1 m above the seabed in a mean current of 22 cm s^{-1} and a Reynolds stress of $2.69 \text{ dynes cm}^{-2}$, showing ejection and sweep phases.

sweep phases have much larger amplitudes than the interaction processes. Comparison of these records with similar laboratory investigations² suggests that the large amplitude events are ejections and sweeps, with an ejection ($w > 0$) preceded by a deceleration of the horizontal flow and a sweep ($w < 0$) occurring after an acceleration.

The distributions of u , w and uw and their departure from gaussian behaviour have been examined in a number of records. The asymmetry of the uw analogue as a result of ejections and sweeps and the net positive input of Reynolds stress, is characterised by a negative skewness of its distribution around the mean. From the distribution of uw in record 2/14 (Fig. 4) it has been possible to determine the contribution of these events to the Reynolds stress. This single record illustrates that events occurring outside -3 standard deviations from the zero stress level contribute as much as 31% in only 3% of the time and events outside -2 standard deviations make a contribution of 57% in 7% of the time. This evidence supports the view¹ that, depend-

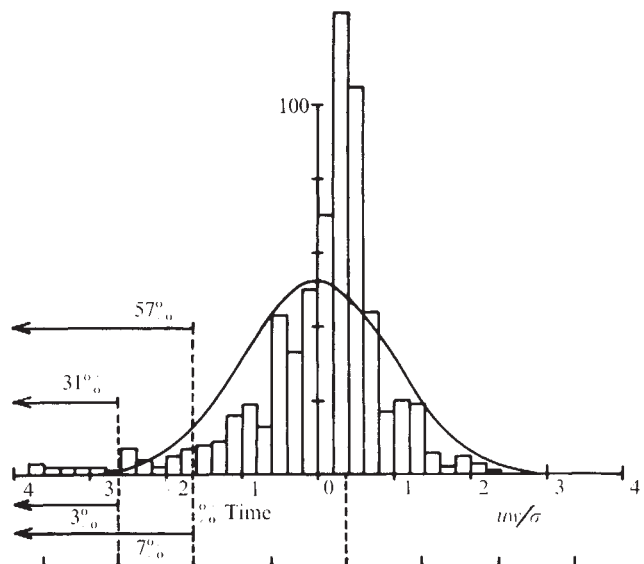


FIG. 4 Distribution of uw in record 2/14 with respect to the mean (upper scale) and zero Reynolds stress (lower scale), showing percentage contributions to uw . Continuous curve is the Gaussian distribution for the same sample and standard deviation. 650 samples; mean $-2.62 \text{ cm}^2 \text{ s}^{-2}$; standard deviation $7.10 \text{ cm}^2 \text{ s}^{-2}$; skewness -1.92 ; kurtosis 9.27 . Percentage contributions to Reynolds stress marked by arrows.

ever, these observations do indicate that the generation of turbulence and Reynolds stress is intermittent and that it is possible to extend the scales of motion involved from laboratory to geophysical proportions.

A. D. HEATHERSHAW

Department of Physical Oceanography,
University College of North Wales,
Marine Science Laboratories,
Menai Bridge, Anglesey

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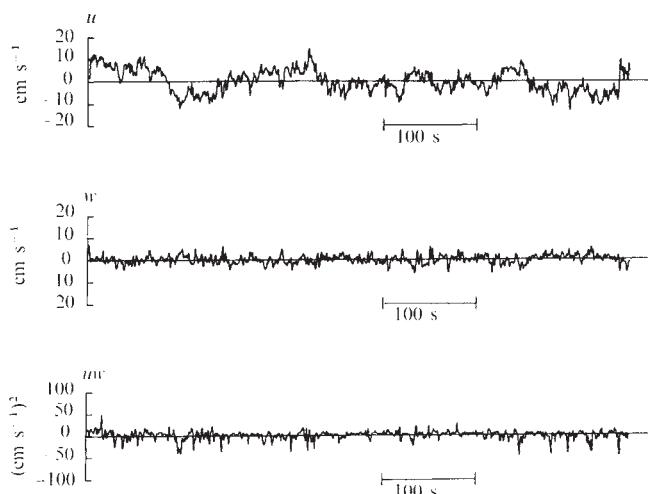


FIG. 3 Bottom turbulence record 11/4/2, station D6, depth of water 40 m. Analogues of u , w and uw at a height of 1.5 m above the seabed for a mean current of approximately 30 cm s^{-1} and Reynolds stress of $3.85 \text{ dynes cm}^{-2}$, showing the intermittent nature of the contributions to uw .

Experiment to measure the antimatter content of the Tunguska Meteor

ON the morning of June 30, 1908 a meteor caused great damage in the region of the isolated trading station Vanovara in Siberia, Russia. Investigations of this meteor, called the Tunguska Meteor, have been described by Krinov¹. Light from the meteor was visible even in a sunlit, cloudless sky. There was an explosive wave, with an energy between 3 and 5×10^{23} erg², blowing down trees over an area of approximately 2,000 km². Thermal energy, estimated to be

TABLE 1 Average chemical composition of continental crust weight %

Element	% (weight)
Oxygen	46.6
Silicon	28.9
Aluminium	8.3
Iron	4.8
Calcium	4.1
Potassium	2.4
Sodium	2.3
Magnesium	1.9
Titanium	0.5
Phosphorus	0.1
Manganese	<0.1

between 1 and 2×10^{23} erg, caused fires and seared trees up to 18 km from the centre of the blast. But it appears as though the meteor never reached the ground, for no crater was formed, nor have any fragments been found which can be positively identified as part of the meteor.

Several explanations have been discussed by Krinov¹. Cowan, Atluric and Libby have suggested that it was an antimatter meteor³ and Jackson and Ryan have postulated that it was a black hole⁴.

Cowan *et al.* suggested that if the Tunguska Meteor were a rock of antimatter, nuclear processes associated with the annihilation of the meteor would have increased the amount of ¹⁴C in the atmosphere. Subsequent assimilation of the ¹⁴C by trees might then be detectable. Their analysis of a tree that had been growing in Arizona in 1909 AD showed a smaller increase in the ¹⁴C content than they had predicted. The significance of this was difficult to interpret because of what seemed to be random yearly variations of ¹⁴C in the local atmosphere. Here I again examine the hypothesis of an antimatter meteor, to ascertain whether other experimental tests can be carried out.

Any event involving nuclear processes should produce radioactive nuclei. Of special interest is the electromagnetic cascade shower. This is produced in air by the decay into γ rays of the π^0 s which are produced when antimatter nuclei annihilate. When an electromagnetic cascade shower containing a wide spectrum of γ rays strikes the ground, radioactive nuclei are produced as a result of many different photonuclear processes.

The major constituents of rock and soil are oxygen, silicon and aluminium with smaller amounts of iron, calcium, potassium, magnesium, phosphorous and sodium, in the amounts given in Table 1 (see ref. 5).

To determine which radioactive products might be produced, the reactions (γ , p), (γ , n), (γ , d), and (γ , 2n) on the various isotopes of the elements listed in Table 1 have been considered. Only a few of these reactions (listed in Table 2) produce nuclei which are sufficiently long lived to be detected now approximately 2×10^9 s after the event.

The reactions which produce ²⁶Al have been given special consideration for two reasons. First, silicon, and to some extent aluminium, are abundant elements in rock (Table 1). Second, ²⁶Al decays predominantly by emitting a positron, whereas ³⁹Ar decays by emitting a β^- ray, and ⁵³Mn by

electron capture. The decay signature of a positron emitter, with the accompanying annihilation radiation, allows the design of systems which will more readily discriminate against background and thus measure smaller total activities.

An experiment to detect the antimatter component of the Tunguska Meteor is thus conceptually simple. The ²⁶Al content of rocks or soil is measured as a function of the distance from the centre of the explosion. The highest concentration of ²⁶Al should be found near the centre. In order to assess the technical feasibility of the experiment, however, the production of ²⁶Al must be examined in more detail.

The number of ²⁶Al atoms, N_{26} , created in the soil and rock by the flux of γ rays is given by the expression

$$N_{26} = \sum_i \frac{A_0}{A_i} f_i \int \frac{dN}{dE} \sigma_i(E) dE \quad (1)$$

where A_0 is Avogadro's constant = 6.02×10^{23} mol⁻¹, A_i is the atomic weight of the nucleus; f_i is the fraction by weight of the nucleus; $\sigma_i(E)$ is the cross section for production for ²⁶Al as a function of photon energy, E , and dN is the number of photons cm⁻² with energy between E and $E + dE$. For the problem under consideration here, equation (1) contains only two terms, corresponding to the nuclei of ²⁷Al and ²⁸Si.

An accurate calculation of the production of ²⁶Al is not possible because of uncertainties in both the incident flux of γ rays and the production cross section of ²⁶Al. Some estimate of these quantities can, however, be made. The γ -ray flux may be estimated in the following way. When a proton and antiproton annihilate, an average of four charged and two neutral pions are produced⁶. Thus about half of the available energy goes into the rest mass of pions and the remainder goes into kinetic energy. Each neutral pion decays into two γ rays which initiate an electromagnetic cascade shower.

The energy density received at the ground is reduced by both the solid angle and absorption in the air. The explosion has been estimated to have occurred between 5 and 6 km up in the atmosphere³. The height of the meteor site is approximately 300 m. At a distance of 5 km, the solid angle subtended by each square centimeter of the ground is 3×10^{-13} sr. The air mass, calculated by using a scale height of 8.4 km and integrating from 0.3 to 5.3 km, is 430 g cm⁻². The energy absorption coefficient of an electromagnetic cascade shower in air is assumed to be the same as that measured for water, that is 0.013 cm² g⁻¹ (ref. 7). This means that approximately 0.37% of the initial energy is transmitted through the air to the ground. The total energy, assumed to be 5×10^{23} erg, or 3×10^{29} MeV, is reduced by a factor of 6 because only a sixth of the energy is converted into γ rays, and by an additional factor of approximately 10^{15} resulting from a combination of the solid angle and absorption. Thus the energy flux at ground level is of the order of 5×10^{13} MeV cm⁻².

The energy spectrum of photons in the cascade shower is uncertain. Because of the kinetic energy of the π^0 s, the initial decay gammas will have a rather broad energy distribution centred at 68 MeV. Processes such as pair production and Compton scattering with subsequent bremsstrahlung by the pair-produced electrons, will further degrade the photon energy spectrum. On the other hand, lower energy photons are more strongly absorbed by the Compton process. (Photoelectric interactions are negligible at the energies considered here.)

Most γ rays produced by cosmic rays and observed in the atmosphere seem to come from π^0 production near the top of the atmosphere⁸. Thus, because the physical process of absorption and creation are similar, the spectrum of the flux induced by the Tunguska Meteor might be expected to be similar to that produced by cosmic rays traversing the same thickness of atmosphere.

If the change in radioactivity is to be detected, the burst of radiation at the Tunguska site must be appreciable when compared with the total cosmic-ray flux integrated over the average lifetime of ^{26}Al . Two estimates of the integrated cosmic-ray flux lead to similar answers. The average radiation background resulting from cosmic rays at sea level is approximately 50 mR yr^{-1} . This implies a total dose of $2.4 \times 10^8 \text{ MeV cm}^{-2} \text{ yr}^{-1}$. The γ -ray flux integrated for energies of about 30 MeV and extrapolated to sea level is approximately $3 \times 10^{-2} \text{ cm}^{-2} \text{ s}^{-1}$ (ref. 8). The integrated energy in this flux yields an energy of $3 \times 10^8 \text{ MeV cm}^{-2} \text{ yr}^{-1}$. The average lifetime of ^{26}Al is $1.07 \times 10^6 \text{ yr}$ and so the additional flux calculated to have been produced by the Tunguska Meteor represents a 15% increase in the total dose received over the past million years.

TABLE 2 Long-lived isotopes

Parent Nucleus	Reaction	Daughter Nucleus	Half-life (yr)
^{27}Al	γ, n	^{26}Al	7.4×10^6
^{28}Si	γ, d	^{26}Al	7.4×10^6
^{41}K	γ, d	^{39}Ar	269
^{64}Fe	γ, p	^{53}Mn	1.1×10^7
^{56}Mn	$\gamma, 2n$	^{53}Mn	1.1×10^7

To obtain the effective production rate, the γ -ray spectrum must be integrated with the cross section as shown in equation (1). The energy spectrum of γ rays in the atmosphere has been measured by Thompson⁸. Very approximately, the energy spectrum of γ rays at a depth of 500 g cm^{-2} can be fit by a power law of the form

$$\frac{dN}{dE} \propto E^{-1.6} \quad (2)$$

The cross sections for the production of ^{26}Al in the ground state by either of the two reactions listed in Table 2 have not been measured. The cross section for $^{27}\text{Al}(\gamma, n)^{26}\text{Al}^*$, where ^{26}Al is the 0.239 MeV first excited state ($\tau_{1/2} = 6 \text{ s}$), has been measured for photon energies up to 62 MeV (ref. 9). If the ^{26}Al is initially formed in a highly excited state, the probability of radioactive decay to the short-lived first excited states with spin and parity of 0^+ should be about the same as for decay to the long lived ground state which has spin and parity of 5^+ . For the predicted spectrum, equation (2), and the measured cross section, the integral evaluated by numerical methods is

$$\int \frac{dN}{dE} \cdot \sigma(E) dE = 3 \times 10^{-15} \text{ photons/nucleus.}$$

Values of the cross section for the second reaction $^{28}\text{Si}(\gamma, d)^{26}\text{Al}$ have not been reported. The cross section for $^{32}\text{S}(\gamma, d)^{30}\text{P}$ has, however, been measured from threshold to 80 MeV (ref. 10). If this measured cross section is used, a numerical value of the integral is obtained.

$$\int \frac{dN}{dE} \cdot \sigma(E) dE = 2 \times 10^{-15} \text{ photons/nucleus}$$

In the following discussion a value of 2×10^{-15} photons/nucleus is adopted for both reactions.

The amounts by weight of silicon and aluminium in average rock are 29 and 8% respectively (Table 1). The production of ^{26}Al in rock at the centre of the meteor site, is therefore estimated to be 4×10^9 atoms per kg of rock. With an ^{26}Al half life of 7.4×10^6 years, this corresponds to an induced specific activity of approximately $10^{-2} \text{ d.p.m. kg}^{-1} \text{ rock}$.

Tanaka *et al.*¹¹ have attempted to measure low levels of activity due to ^{26}Al found in deeply buried rock. They report an upper limit of $10^{-2} \text{ d.p.m. kg}^{-1} \text{ rock}$, a value

which is approximately the size of the predicted effect.

Most detection systems for measuring very low levels of positron emission consist, at least in part, of opposing NaI(Tl) crystals which detect in coincidence the two 0.511 MeV γ rays from the annihilation of the positron^{12,13}. For such a system, and for a given observing time, the sensitivity, S , is defined as the ability of a detector to measure activity, and

$$S \propto \frac{\epsilon N}{\sqrt{B}} \quad (3)$$

where ϵ is the detector efficiency, N is the number of nuclei that can be placed in the detector and B is the background rate. Tanaka *et al.*¹¹ suggested that the sensitivity of their instrument could be improved if larger detection crystals were used and if purer and more concentrated samples were obtained. These ideas suggest that considerable increase in sensitivity should be possible. For example, NaI(Tl) crystals are available with an area up to 100 times that of detectors used by Tanaka *et al.*¹² and Roedel¹³, and three times as thick. Even if the background increases linearly with the volume of detector, sensitivity would be increased by a factor of six because more sample could be placed between the crystals. In addition, such large NaI(Tl) would increase the photo-fraction efficiency and thus the sensitivity.

The use of pure aluminium, rather than the Al_2O_3 used by Tanaka *et al.* would increase the reported sensitivity by a factor of about two because more Al nuclei could be introduced into the same volume.

It might be possible to select rocks, such as quartz or limestone; which are largely free from aluminium. The extraction of aluminium from such rock samples would increase the specific activity of ^{26}Al in the sample by more than an order of magnitude.

In addition to the ideas discussed above, a promising method of reducing the background in β^+ counting systems, by observing the positron in coincidence with the annihilation γ rays, has been developed¹⁴. We have recently developed a simple version of such a system which yields a gain in sensitivity of a factor of two over an equivalent two- γ -ray detector system.

With the technical improvements in detectors that seem feasible, a system with an increase in sensitivity of almost two orders of magnitude could be planned. This would allow the detection of an increase of only a few % in ^{26}Al content. The proposed experiment could then be readily carried out.

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HALL CRANNELL*

Department of Physics,
The Catholic University of America,
Washington DC 20017

Received November 12, 1973.

* Present address: Department of Physics, Westfield College, London NW3 7ST.

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Comets, solar wind and the D/H ratio

I WISH to speculate on the consequences of considering together two astronomical observations. First, the Sun emits a matter flux of $\sim 10^{12}$ g s⁻¹ in the form of a solar wind¹. The chemical composition seems to be rather similar to the solar photospheric composition². So it incorporates a fractional mass of about 1% of atoms which are good candidates for grain formation (ices as CH₄, NH₃, H₂O; dusts as silicates and irons). Thus a flux of $\sim 10^{10}$ g s⁻¹ of condensable matter flows away in the remote parts of the Solar System.

Second, the number of newly arriving comets (of long period) is about one a year with a mean mass of $\sim 10^{18}$ g (ref. 3) and a comet can be pictured plausibly as a conglomerate of ices and dusts⁴. Thus a mean flux of $\sim 3 \times 10^{10}$ g⁻¹ of cometary matter flows inwards from the remote part of the Solar System.

These fluxes seem to agree within one order of magnitude or better. I assume that this is not a pure coincidence.

The process could be pictured in the following way. The ionised atoms of the solar wind follow the laminar flow lines until they reach a boundary where, presumably because of interaction with the interstellar magnetic field, the wind becomes turbulent. There, they are progressively decelerated, recombine, form molecules and eventually grains of increasing sizes. (Although very little is known about how the grains are formed, observations of interstellar matter indicate that the process must be very efficient; otherwise, how could the striking depletion of the refractory elements in the interstellar gas^{5,6} be explained?) The grains are carried away by the turbulent gas and collide with each others. Dust and ices will mix and stick together, thus generating the embryos of comets.

These objects are subjected to two forces: the inward gravitational pull of the Sun and the drag force of the turbulent motions with its residual outward component. As the size of the object increases the second force becomes gradually weaker than the first. The outward motion is decelerated until the object start moving toward the Sun. A comet is born.

So we have a picture of a stationary cloud in which matter is fed in through the solar wind, and out through conglomerates of ices and dusts. The cloud has, in the past, grown to a stage at which the feeding out just compensates the feeding in, thereby explaining the rough equality between the two fluxes.

Before this model becomes credible several points will have to be clarified, several quantitative estimates (of the location and properties of the cloud, of the accretion rate in the cloud and so on) will have to be carried out and several 'tests' will have to be 'passed.' For example, the observed distribution of kinetic energies, of ellipticities and of orbital inclinations of the comets should find a reasonable explanation within the frame of the model.

The orbital planes of the long-period comets do not seem to raise any problem: they are tilted in all directions as expected from the plausible spherical symmetry of the solar

wind. The eccentricities would be assigned to such effects as the random drag velocities of the cells.

But at present, instead of pushing this discussion further, I stress the importance of measuring the D/H ratio in cometary matter by showing its relevance to the problem of the origin of the comets.

The D/H ratio in interstellar space is 1.5×10^{-5} (ref. 7). In oceanic and meteoritic water, on the other hand, the ratio is 1.4×10^{-4} ; the increase is probably due to molecular exchange reaction at the time of formation of the water⁸. In the solar wind D/H $< 3 \times 10^{-6}$, (ref. 9) compatible with the fact that the Sun burned its deuterium in its early days.

Clearly this model of comet formation would predict essentially no D (D/H $\ll 10^{-5}$). If, on the other hand, the comets were made early in the history of the Solar System, by matter left over from the protosolar condensation (the Oort's cloud), depending on their formation temperature we should have in the various cometary molecules a D/H ratio of anything from 10^{-5} to 10^{-4} and possibly even more (see tables in ref. 10). Measurements of DCN/HCN, CH₃D/CH₄, OD/OH in comets would be of the utmost importance.

HUBERT REEVES

SEP-CENS, Saclay 91 Gif-sur-Yvette, France

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Evolution of dense galactic nuclei through dwarf star collisions

STELLAR collisions are important in dense galactic nuclei¹⁻³. QSOs, N galaxies and Seyfert galaxies release energy on similar short time scales⁴⁻⁷ but detailed models of stellar collisions have been limited so far to collisions between two main sequence stars, with the conclusion that for an initial relative velocity $V_{rel} \lesssim 10^3$ km s⁻¹, and small impact parameter, coalescence follows. At $V_{rel} \sim 5,000$ km s⁻¹ two colliding main sequence stars are largely disrupted and a non-negligible amount of relativistic particles is formed⁸. Here we draw attention to the fact that a very important type of collision in dense galactic nuclei is one in which a white dwarf (WD) is involved.

Van den Bergh³ has pointed out that most collisions in the nuclei of 'normal' galaxies such as M32 involve two dwarf M stars or one M dwarf (dM) and one late-type giant. The reason is the high abundance of dM stars and the large cross section of giants. The evolution of a nucleus therefore depends on the outcome of WD-WD, WD-dM, dM-dM and dM-giant collisions. We neglect dM-giant collisions because most of them will occur in the highly rarified atmosphere of a giant and consequently will have a very small effect. For simplicity we discuss only dM-WD collisions with impact parameter p smaller than $0.75R$, where R is the radius of the red dwarf.

The escape velocity of a white dwarf varies from 2,500 km s⁻¹ for a WD with $M = 0.25M_{\odot}$ to 16×10^3 km s⁻¹ for a WD with $M = 1.25M_{\odot}$; it is ~ 600 km s⁻¹ for late-type main sequence stars. Consequently, the WD-dM collision of low V_{rel} is governed by the white dwarf gravitational field. As the initial relative velocity of the colliding stars increases (the stellar cluster becomes denser) the dynamics of the collision are governed more and more by the initial relative velocity.

We consider a typical collision between a WD of $1M_{\odot}$ and a dM star of $0.2M_{\odot}$ at $V_{rel} \sim 10^4$ km s⁻¹ and impact parameter $p < 0.75 R$. Since this velocity is greater than the speed of sound in the dM star the effect of gravity on the structure of the red dwarf is small. Further, the change in the velocity of the WD during the collision can be neglected. The time spent by the WD inside the dM star is 10 to 30 s which is more than an order of magnitude smaller than the dynamical time scale of the dM star. So only a small amount of mass can be accreted onto the WD from the dM star. Hoyle and Lyttleton⁹ found that the "capture radius" σ of a star of mass M moving at constant velocity V through an infinite medium is $\sigma = 2GM/V^2$, where G is the gravitational constant. In our example $\sigma \sim 3 \times 10^8$ cm and the density scale height h_p for the dM is $\sim 10^9$ cm for $r \lesssim 0.75R$. So the assumption of an infinite medium is valid. But σ is about $\frac{1}{2}$ the radius of the WD and Hoyle and Lyttleton's theory is not valid for $\sigma < R_{WD}$. A plausible assumption is that $\sigma \sim R_{WD}$ and the total mass accreted by the WD is then $(1 \text{ to } 3) \times 10^{-4}M_{\odot}$. All other parameters being equal, the total mass accreted by a WD will increase for white dwarfs with lower mass and larger radius.

Most of the accreted material is rich in hydrogen because nuclear timescales in a $0.2M_{\odot}$ star are longer than the Hubble time. When the mass accreted exceeds $\sim 10^{-5}M_{\odot}$ nuclear energy generation becomes violent enough to drive a thermal runaway¹⁰⁻¹³ typical of novae. The fate of the runaway depends on the WD initial temperature T (ref. 13). At $T \lesssim 3 \times 10^7$ K the runaway ends in an explosion whereas in hot white dwarfs with $T \gtrsim 4 \times 10^7$ K the energy generation rises until a steady state is reached. At some later time the hydrogen is exhausted and the nuclear reaction decays. In models calculated by Saslaw¹³ 10^{47} to 10^{48} erg is released. It will be shown later that plausible models of galactic nuclei give time intervals, between subsequent collisions, of 10^6 to 10^7 yr for each white dwarf. Since the cooling time of white dwarfs is $\sim 10^9$ yr most white dwarfs have $T > 4 \times 10^7$ K and the second process mentioned above occurs. The typical white dwarf thus produces a series of novae at a rate of one per 10^6 to 10^7 yr.

Similar arguments concerning collision energetics show that most dM-dM collisions lead to substantial mass loss if $V_{rel} \sim 10^4$ km s⁻¹, and that the rarer head-on collisions disrupt both stars. Thus, dM-dM collisions transform the dM stars into white dwarfs made of unburnt nuclear material.

In the case of WD-WD collisions a simple estimate yields a collision cross section $\sigma_{WD-WD} \sim (500)^{-1} \sigma_{WD-dM}$ for $V_{rel} \sim 10^4$ km s⁻¹. At $V_{rel} \sim 10^4$ km s⁻¹ the most probable outcome of a collision of two white dwarfs of $1M_{\odot}$ is one white dwarf with a total mass in the range 1 to $2M_{\odot}$. Such a velocity is too low to be energetically capable of disrupting the stars. The WD-WD collisions differ from the WD-dM collisions in two respects. First, WD-WD collisions raise the mass of the remaining white dwarf more than a WD-dM collision and may bring the final product to a mass above the Chandrasekhar limit. Second, the frequency of WD-WD collisions is two or three orders of magnitude smaller than the frequency of dM-WD collisions. This will probably give rise to supernova explosions of a certain frequency.

The evolution of a dense galactic nucleus depends on the transformations between the various stellar species due to stellar collisions. We consider a highly simplified model of a

spherical galactic nucleus of radius R made up of N_{dM} dM stars of $0.2M_{\odot}$ and radius $r_{dM} \sim 0.2R_{\odot}$, and N_{WD} white dwarfs of $1M_{\odot}$ and radius $r_{WD} \sim 8 \times 10^{-3}R_{\odot}$.

We assume that the average velocity can be obtained from the Virial Theorem. The mean free path of a white dwarf for collision with a dM star is

$$\lambda_{WD-dM} \sim \frac{R^3}{N_{dM} r_{dM}^2}$$

and similar expressions hold for λ_{dM-dM} and λ_{WD-WD} . The total number of WD-dM collisions per unit time, n_{WD-dM} is given by

$$n_{WD-dM} \sim 3 \times 10^{16} N_{WD} N_{dM} M_i^{1/2} R^{-7/2} \text{ s}^{-1}$$

where M_i is the total mass of the nucleus and we assume roughly equal velocities for all stars.

We consider the following ranges for the various parameters: $N_{dM} = (0.2 \text{ to } 20) \times 10^{10}$, $N_{WD} = (0.2 \text{ to } 20) \times 10^{10}$ and $R = (0.25 \text{ to } 1.0)$ pc (see refs 14 and 15) in which the parameters of NGC4151 are discussed). Two fairly extreme cases are $R = 0.25$ pc, $N_{dM} = 2 \times 10^{10}$ and $N_{WD} = 2 \times 10^{11}$; and $R = (0.25 \text{ to } 1.0)$ pc (see refs 14 and 15 in which the typical energy releases for novae are $\sim 10^{47}$ erg then $\sim 10^{48}$ erg s⁻¹ and $\sim 10^{42}$ erg s⁻¹ are released in these two models. The supernova rate varies from 10^3 yr⁻¹ to 10^{-3} yr, respectively. But most of the contribution to the total energy release does not come from the supernovae.

Eventually, the number of dM stars decreases and with it the total energy output. If one starts with $N_{dM} \sim 2 \times 10^{11}$ (all of $\sim 0.2M_{\odot}$) and $N_{WD} \sim 2 \times 10^{10}$ (all of about $1M_{\odot}$) the energy output matches that of bright QSOs. A reduction of N_{dM} by a factor of 10 (which is caused by dM-dM collisions over a timescale of 10^6 yr) brings to total energy output to the range of N-type galaxies. Still another reduction by a factor of 10 (over a timescale of 10^8 yr) reduces the energy output to the range of Seyfert galaxy nuclei. Finally, in about 10^9 yr N_{dM} drops to $\sim 2 \times 10^6$ and the energy output due to collisions falls to about 10^{40} erg s⁻¹. The timescale for the change in N_{dM} is easily obtained from the collision frequency and average mass loss per collision. Clearly, the timescale for changes becomes longer as the number of dM stars decreases. At the same time the principal energy source changes as well. At early stages it is predominantly the dM-dM collisions whereas later it becomes the WD-WD collisions and finally the WD-dM collisions.

So it seems that, assuming about 10^{47} erg to be released in a nova caused by a WD-dM collision, we find that stellar collisions in dense galactic nuclei can explain the basic energetics of QSOs, N-type galaxies, Seyfert galaxy nuclei and the central regions of bright elliptical galaxies, as well as their evolutionary connection and sequence.

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M. M. SHARA
G. SHAVIV

Department of Physics and Astronomy,
Tel-Aviv University, Tel-Aviv, Israel

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Observation of $O^+(^2P^o-^2D^o)\lambda 7,319\text{\AA}$ emissions in the twilight and night airglow

TEN years ago Dalgarno and McElroy¹ suggested that the $^2P^o \rightarrow ^2D^o$ transition of the atomic oxygen ion would be produced in the upper atmosphere through the simultaneous photoionisation and excitation of atomic oxygen atoms by solar ultraviolet radiation.

The photoionisation cross section calculations of Dalgarno, Henry and Stewart² indicate that approximately 25% of the photons absorbed by atomic oxygen below 663 Å will produce O^+ ions in the metastable $^2P^o$ state. Using these cross sections, Dalgarno and McElroy³ computed dayglow emission rates and later extended their calculations to the twilight situation⁴. This excitation mechanism has been observed in the laboratory in photoelectron spectroscopy measurements by Jonathan *et al.*⁵ who found an efficiency of ~22% for producing the $^2P^o$ state at 584 Å. Thus, both theory and experiment indicate that photoionisation excitation of $O^+(^2P^o)$ is an efficient process and should produce an observable $^2P^o \rightarrow ^2D^o$ airglow feature as predicted in the work of Dalgarno and McElroy.

As yet no observations of this transition in the airglow have been reported.

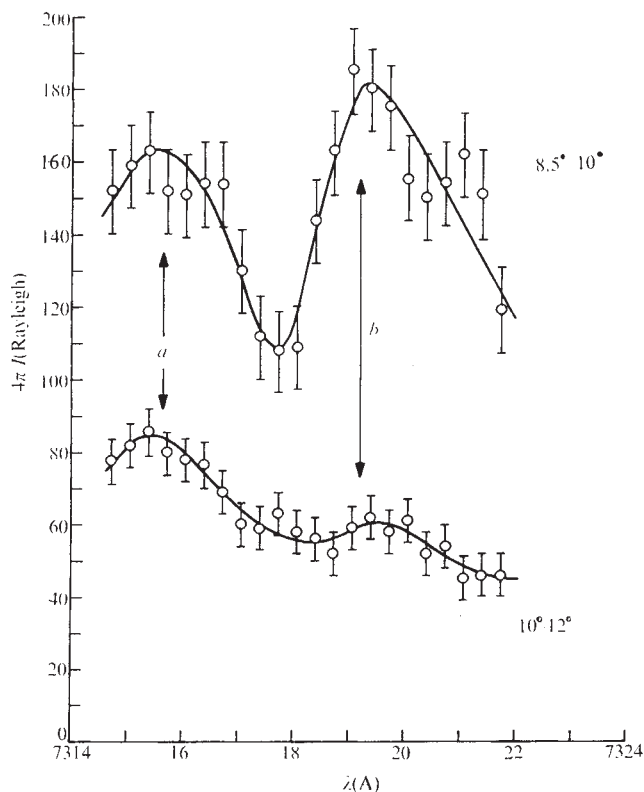


FIG. 1 Upper spectrum: average of the observations obtained during evening twilight for solar depression angles in the range 8.5° to 10° . Lower spectrum: evening scans between 10° and 12° . Points shown represent three-point running averages. a, OH (8-3) $P_1(2)$ feature; b, $O^+ \Pi (^2P^o-^2D^o)$ feature. August 26-30, 1973.

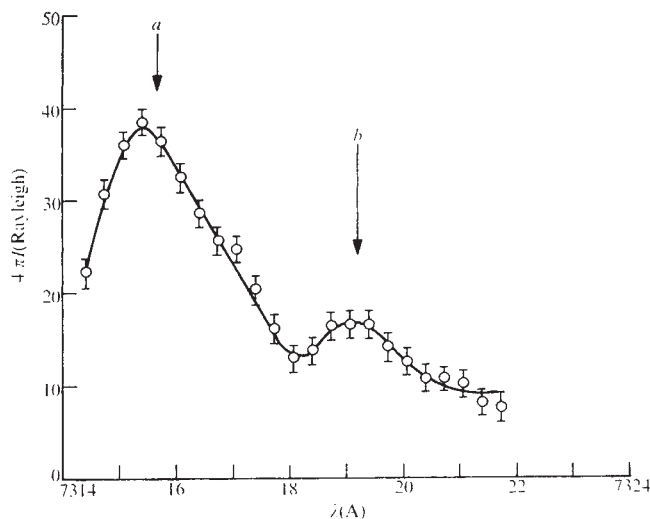


FIG. 2 Observed night airglow spectrum. a, b as Fig. 1. August 26-30, 1973.

This work reports twilight and nightglow observations of the $^2P^o_{1/2,3/2}-^2D^o_{5/2}$ line group at $\lambda 7,319\text{\AA}$. Assuming that the upper J levels are populated according to their statistical weights, then the $^2P^o_{3/2}-^2D^o_{5/2}$ $\lambda 7,319.4\text{\AA}$ line will be stronger by approximately a factor of three or greater than the $^2P^o_{1/2}-^2D^o_{3/2}$ $\lambda 7,318.6\text{\AA}$ line. The mean wavelength for the two lines is then approximately $7,319.2\text{\AA}$. The observations were made in the zenith at Table Mountain which is ~70 km NNE of Los Angeles and 2,300 m above sea level. The sky conditions there are excellent for airglow observations. A 0.3 m $f/5.3$ spectrometer with a $1,200\text{ lines mm}^{-1}$ grating blazed at $5,000\text{\AA}$ was used to disperse the spectrum. The entrance and exit slit sizes were $55\mu\text{m} \times 2\text{ cm}$ and $65\mu\text{m} \times 2\text{ cm}$ respectively, which resulted in a spectral resolution of approximately $2.3\text{\AA}$. Any second order contribution was reduced using two interference filters, one in reflection and one in transmission. The radiation was detected with an EMI 9558 QB photomultiplier with an S-20 photocathode cooled to -20°C . Using magnetic and optical lenses, only the central portion of the photocathode was effective, resulting in a dark counting rate of $1-2\text{ s}^{-1}$. The observations were obtained during the period of new Moon, August 26-30, 1973. The wavelength region $7,314$ to $7,322\text{\AA}$ was scanned repeatedly at $5\text{\AA min}^{-1}$ during twilight and accumulations for 4 s were recorded. The nightglow measurements were obtained at slower scanning speeds and longer integration periods.

The Rayleigh scattering component of the twilight was estimated by least-squares fitting and extrapolation of the signals observed for solar depression angles between 4° and 8° . This contribution was subtracted from the observed signals, with the dark count signal, to give the spectra shown in Fig. 1.

It is clear from Fig. 1 that the $O^+(^2P^o-^2D^o)\lambda 7,319\text{\AA}$ feature is present in the twilight spectrum and decreases in intensity as the solar depression angle increases, in qualitative agreement with the variation expected for excitation by solar ultraviolet radiation. The absolute intensity of the O^+ feature is greater than that predicted by Dalgarno and McElroy. This may be due to both experimental error and differences between the actual and model atmosphere and the absorption cross sections used in the calculations⁴. The nearby OH (8-3) $P_1(2)$ line⁶ is also apparent and seen to diminish in brightness as evening twilight progresses approximately as predicted by the model of Shimizaki and Laird⁷.

In the nightglow, where direct solar excitation is absent, the O^+ feature is still found (Fig. 2). We suggest that extreme ultraviolet photoionisation excitation is also responsible for

at least part of this nightglow feature. Both He I $\lambda 584$ Å and He II $\lambda 303$ Å emissions will be incident on the night-time upper atmosphere, arising from resonance scattering of the solar helium lines, the former by interplanetary and exospheric He and the latter by plasmaspheric He⁺ ions⁸⁻¹². It is difficult to estimate the nightglow $\lambda 7,319$ Å intensity that would be produced by this excitation mechanism since the background ultraviolet radiation can show considerable variation. The He I $\lambda 584$ Å may exhibit a seasonal variation due to the non-uniform interplanetary density distribution¹³ and plasmaspheric He⁺ concentrations, and the resulting $\lambda 303$ Å flux, can also show large fluctuations.

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R. W. CARLSON
K. SUZUKI

Department of Physics,
University of Southern California,
Los Angeles, California 90007

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An organic 'metal'?

LITTLE¹ has proposed a mechanism by which an electron-electron (e-e) interaction in certain organic polymers and macromolecules might result in a high superconducting transition temperature. This e-e mechanism requires a combination of two systems consisting of a conducting or semiconducting polymeric compound forming a spine, with highly polarisable side chains attached to the spine. According to Little, the addition of such side chains can increase the e-e attraction in the spine to the point where it becomes energetically favourable to enter the superconducting state. This e-e mechanism of superconductivity has led to lively discussions, primarily concerned with superconductivity in quasi one and two-dimensional systems^{2,3}, and the magnitude of the e-e attraction^{4,5}. It has been shown theoretically³ that, depending on the nature of the interaction between the electrons, a quasi one-dimensional system may exist in different states: metallic, dielectric, antiferromagnetic and superconducting. It has also been shown experimentally that the organic charge transfer salt N-methyl-pehnazinium tetracyanoquinodimethan has 'metallic' properties at temperatures above 200 K with a continuous transition to a small-band-gap insulator below 200 K (ref. 6).

We have been investigating a number of organic compounds in which the e-e interaction is expected to be large. Here we wish to report one of a potentially large class of organic compounds which has 'metallic' electrical properties over a large temperature range. This compound, we believe, is an intercalation compound consisting of a triphenylmethane dye (malachite green) and graphite. Graphite in which the bonding between the carbon layers is weak has been known to form intercalation compounds with alkali metals⁷, acids⁸, halogens⁸ and metal chlorides⁹. The intercalation compounds of K, Rb, and Cs with graphite have been shown to have superconducting transition temperatures of 0.55, 0.155, and 0.135 K respectively¹⁰. (Metallic conduction has also been observed in the metal chloride intercalation compounds⁹.) In the complex described here the graphite, which is a highly conjugated layer compound, acts as the semiconducting spine or sheet, and the dye, complexed to the graphite, is similar to the polarisable side chains of Little's model. The chemical structure of the malachite green dye molecule, and two of its resonance structures is shown in Fig. 1.

This complex, and others of the same general class, is prepared as follows: very high purity samples, 98-99% pure, of malachite green dye and powdered graphite are thoroughly mixed in a mass ratio of three parts of dye to one part of graphite. The powder mixture is heated to the melting point of the dye. The melting point of the dye depends on the purity of the sample and is, in general, greater than 250° C. When the entire sample is in a soft to semi-molten state, the complex is allowed to cool to room temperature when it becomes a hard, black vitreous solid.

Powder X-ray diffraction studies of this compound have shown a broad diffraction peak equivalent to a lattice spacing of 6 Å which may indicate the formation of an intercalation complex. Probes for resistance measurements were usually inserted into the complex while in the soft state, at 150-200° C and the complex was allowed to cool. On cooling, the probes became tightly bound to the sample. Resistance measurements were usually made with copper probes, 1 cm long, separated by distances of 0.4 cm.

Figure 2 is a plot of resistance against temperature for the dye-graphite complex between 77 and 333 K. Between 77 and 250 K, the resistance is seen to be proportional to the temperature, with a slope of 0.1 ΩK⁻¹. At temperatures above the softening point (40 C-60° C), the curve again becomes linear, with a slope of 22 ΩK⁻¹. Furthermore, four-probe resistivity measurements have shown the resistivity to have the same dependence on temperature as the resistance. Unlike most organic complexes based on Little's model¹¹, this complex is rather unique in that over the temperature ranges investigated, there seems to be no activation energy. Furthermore, between 77 K and 250 K the proportional relationship between resistance and temperature is not unlike that for metals at temperatures greater than the Debye temperature. It should be stressed that this complex does not necessarily form a stoichiometric ratio. Resistance measurements indicate that the graphite is gradually converted from a semiconducting state to a 'metallic' state by

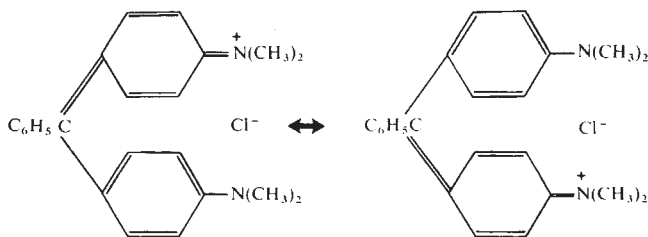


Fig. 1 Molecular structure of malachite green dye showing the two resonance structures which correspond to a positive formal charge on the two amine groups.

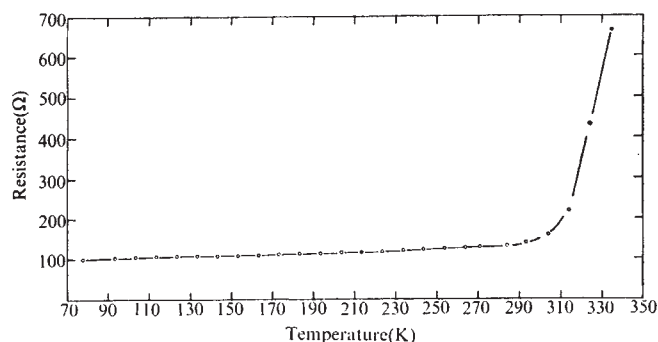


FIG. 2 Plot of resistance against temperature for malachite green dye-graphite complex.

increasing dye concentrations. Further increased dye concentrations convert the graphite back to the semiconducting state.

If the conductive properties of this complex result from an e-e interaction, the fact that it does not exhibit a 'high' temperature superconducting state, if a superconducting state at all, is an extremely complicated problem. Malachite green has an absorption band in the region 600–700 nm. Calculations¹² show that it is necessary to have more polarisable dyes, with absorption bands in the region of $\sim 1,200$ nm, to induce a sufficient e-e interaction in the spine. Furthermore, it is expected that the e-e interaction depends strongly on the distance separating the dye and the spine. For large separations, the interaction decreases rapidly¹² whereas for short separations (strong interaction) exchange interactions can occur between the conduction electrons in the spine and the dye molecule. Such exchange interactions could limit the strength of the e-e interaction in the spine, by the conversion of the dye to a 'stable', less polarisable radical. Nevertheless, if further measurements confirm the 'metallic' state of this complex, or class of complexes, it would indicate that the Coulomb interaction between localised electrons in the conjugated graphite sheet is greatly reduced. Such screening of the Coulomb interaction by the dye would then allow electron delocalisation to the extent that a 'metallic' state results.

We believe that this complex is only one of a rather large class of similar compounds based on the intercalation of triphenylmethane dyes (general class) and 'layer type' semiconductors and conductors. Examples of such 'layer type' semiconductors include graphite, MoS_2 , and BN ; whereas, TaS_2 and WS_2 are examples of 'metallic' layer compounds. Results of further detailed investigations concerning these compounds in the polycrystalline and single-crystal state will be published elsewhere.

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E. J. SEYKORA

Department of Physics,

R. A. KLEIN

Department of Chemistry, East Carolina University,
Greenville, North Carolina

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Rotation of the geomagnetic field

THE geomagnetic field apparently drifts westward about the geographic axis at about $0.2^\circ \text{ yr}^{-1}$. Malin & Saunders have attempted to determine the directions of the axes about which a 'westward' drift would best fit the fields during six successive epochs¹. They did not determine the circular standard deviations of the axis directions but, as the five successive axes moved fairly smoothly through an arc of about 20° , they assumed that the axes had been reasonably well determined, and interpreted the movement in terms of physical processes in the Earth's core.

The axes they determined were, however, almost certainly not those appropriate to their interpretation, and it is unlikely that the correct axes can be determined to a useful accuracy.

They made a numerical search for that combination of axis and drift which maximised the correlation between a function f , specified on the surface of a sphere at one epoch, and the corresponding function F five years later; that is, they looked for that combination which maximised the mean value of f^*F over the sphere, where f^* is the rotated version of f . This process is in fact exactly the same as searching for that combination of axis and drift which minimises the mean square of the difference $(F - f^*)$, a problem in least squares. This, in turn, is exactly the same as fitting the difference $\Delta F \equiv (F - f)$ by the difference $\delta f \equiv (f^* - f)$ by least squares, that is, fitting the integrated secular variation by the 'westward' drift of the field.

The functions f and F were specified in terms of a finite series of coefficients, g_n^m , h_n^m and G_n^m , H_n^m , for $n = 2$ to 6, of (semi-normalised) spherical harmonics defined with respect to the geographic axis. Correspondingly ΔF can be specified in terms of coefficients ΔG_n^m , ΔH_n^m , and, after some manipulation, δf in terms of δg_n^m , δh_n^m . Because the spherical harmonics are orthogonal over the sphere, minimising the mean of $(\Delta F - \delta f)^2$, is the same as minimising the sum

$$S = \sum_n \alpha_n \sum_{m=0}^n [(\Delta G_n^m - \delta g_n^m)^2 + (\Delta H_n^m - \delta h_n^m)^2], \quad (1)$$

where α_n is the mean square value over the sphere of the function produced by a unit amplitude harmonic n , m ; because of the normalisation, α_n is independent of m .

The axis of drift may be obtained from the geographical axis by a rotation having components $(u, v, 0)$ in the $\lambda = 0^\circ$, $\lambda = 90^\circ$, and $\theta = 0^\circ$ axes respectively, and the westward drift rate about the new axis may be denoted w . For convenience the westward drift $[(u, v, 0); w\delta t]$ may be denoted by Ω .

To perform the rotation of axes, Malin and Saunders used the Taylor series method of James². As u and v were at most 14° , and $w\delta t$ was about 1° , it is sufficient to take the series to second order for the following discussion. This shows that, for example,

$$\delta g_n^m = w \delta t (m h_n^m + b u^2 + c u v + d v^2), \quad (2)$$

where b , c , and d are linear combinations of the coefficients $g_n^{m'}$, $h_n^{m'}$, with $m' = m - 2, m - 1, m, m + 1, m + 2$, but with all the terms having the same value of n . Thus while the secular variation produced by the drift is proportional to w , the first order terms in u and v have cancelled. This confirms the intuitive result that the effect of a small 'westward' drift is going to be very insensitive to a small change of drift axis. It follows that the values found for u and v will be much more uncertain than those for w . Intuitively, if w is uncertain by, say, 10%, then we would expect u and v to be uncertain by about 0.1 rad, about 6° .

For a given epoch, the problem of estimating the best values of the parameters u , v , w by least squares can now be considered, that is, the minimisation of the sum S of (1). This corresponds to fitting a 'dependent' variable by a function of an 'independent' variable, the function having known form but involving the parameters which are to be determined. The coefficients of the integrated secular variation, ΔG_n^m , ΔH_n^m , are the values of the 'dependent' variable and, although specified, they are subject to considerable error. They are to be fitted by δg_n^m , δh_n^m , which are complicated functions of the values g_n^m , h_n^m of the 'independent' variable (which in comparison are effectively exact), and of the parameters u , v , w , which are to be estimated. The resulting estimates of these parameters will be subject to errors, which will increase as the errors in ΔG_n^m , ΔH_n^m become larger. As the rotation of axes does not introduce any terms of different n , the separation of S into contributions of different n is still possible. If these contributions were separately minimised five different values of Ω would be obtained, which can be called Ω_n . (Richmond³ showed that, for drift about the geographic axis, the w_n ranged between 0.01 and $0.23^\circ \text{ yr}^{-1}$.) Malin⁴ estimated the standard deviations of ΔG_n^m , ΔH_n^m used by himself and Saunders¹. The fractional errors were about 10% for $n = 2$, increasing to about 50% for $n = 6$. Clearly the Ω_n would be subject to considerable errors, larger with increasing n . (For drift about the geographical axis it can be shown, using conventional error theory, that Malin's figures would give standard deviations of w_n varying from 0.01 to $0.04^\circ \text{ yr}^{-1}$; the effect of introducing the other two parameters u and v is probably small, but will always increase these values.)

Because of the cancellation in (2) of the first order terms in u and v , the least squares problem is non-linear even to the first approximation, and it is not possible to perform an analysis of its solution. The α_n parameters in (1), however, clearly behave as 'weights' in the least squares solution; if one of the α_n parameters were considerably larger than the others then Ω may be expected to depend mainly on that Ω_n .

In the discussion of their results, Malin and Saunders interpreted Ω as the angular motion of the outer layers of the electrically conducting fluid core. The magnetic lines of force in the core are probably 'frozen' to the fluid on the short time scales of the secular variation, and the interpretation of Malin and Saunders would be reasonable if the functions they had correlated were the radial magnetic fields at the surface of the core. It appears, however, that what they did correlate were the magnetic potentials at the surface of the Earth. For a unit amplitude harmonic n , m , the mean square potential on the Earth's surface is $1/(2n + 1)$, whereas the mean square radial field is $(n + 1)^2/(2n + 1)$, and the extrapolation down to the core gives another factor of $(1.83)^{2n}$; the α_n used by Malin and Saunders over-weighted the $n = 2$ harmonics by about 700:1 compared with the $n = 6$ harmonics. This would not have mattered if the field rotated bodily without changing its pattern, but different harmonics do have different drift rates, and the drift contributes only about half of the secular variation. Rich-

mond⁵ has shown the large differences in w which result from using these different weightings. Therefore the Ω obtained by Malin and Saunders are almost certainly not those appropriate to their interpretation.

Because of the very high weighting of the harmonics of low degree, the fairly smooth variation of Ω is probably mainly an indication of the fairly smooth, and comparatively well determined, changes of the small number of $n = 2$ coefficients. Their weighting also means that Ω will have smaller errors than the correct Ω ; for drift about the geographic axis, and using the standard deviations of Malin⁴, their weighting gives a standard deviation of w of about $0.005^\circ \text{ yr}^{-1}$, whereas the correct weights give about $0.022^\circ \text{ yr}^{-1}$, corresponding to about $1\frac{1}{2}^\circ$ and 7° respectively for the axis directions. So a recalculation of Ω using the correct weights would be expected to give a much bigger scatter of the drift axes, and make any movement of the axes much more difficult to determine.

But using coefficients up to only $n = 6$ to calculate Ω does not in any case give a reasonable approximation to the rotation of the core surface. The contribution of the secular variation coefficients of a given degree, n , to the mean square field at the core surface forms one term of a series which shows no sign of convergence up to $n = 6$, and the contribution of the higher coefficients is not known⁵.

F. J. LOWES

School of Physics,
The University,
Newcastle upon Tyne NE1 7RU, UK

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DRS MALIN AND SAUNDERS REPLY: Lowes questions our conclusion on the grounds that the pole positions are not sufficiently well determined to show significant departures from the geographical pole.

In the absence of a satisfactory method for estimating the standard errors of the pole coordinates (Lowes gives an intuitive figure of about 6° , whereas our intuition, based on consistency of results, would give a smaller figure), we will attempt to test the validity of our results by the traditional method of a 'crucial experiment'. If our results are valid, they would predict that the optimum pole positions for epochs before 1940 would lie near the magnetic pole-Novaya Zemlya line and that their distance from the geographical pole would increase with the remoteness of the epoch. Further, we would expect the rotation rate to be smaller for earlier epochs. Alternatively, if the pole of rotation is really the geographical pole, we would expect the earlier optimum pole positions to be randomly distributed about the geographical pole.

We selected a uniform set of spherical harmonic models for the epochs 1905, 1915, 1925, 1935, 1945, derived from the main field and secular change models of Vestine *et al.*¹ and listed by Barraclough². The models are to the sixth degree and order, and are based on grid-point values of North and East magnetic intensity read from charted values of survey, repeat station, and observatory data. Although these data are less uniformly distributed with time than those used in our previous study, they are the best available at present. The method of determining the optimum pole positions and rotation rates is the same as that which we used previously³, and the results are given in Table 1. The results for 1925-1935 are in parenthesis because the rotation

TABLE 1 Pole positions and rotation rates required to give maximum correlation between magnetic field models for different epochs

Epochs	Mean epoch	Interval T (yr)	Pole position		Rotation δ/T (degrees yr ⁻¹)
			E. Longitude λ' (degrees)	Co-latitude, θ' (degrees)	
1905-15	1910	10	65.2	82.4	0.134
1905-25	1915	20	62.4	77.9	0.125
1915-25	1920	10	58.5	72.8	0.114
1905-35	1920	30	60.0	72.5	0.111
1915-35	1925	20	55.7	65.5	0.093
1905-45	1925	40	56.3	65.4	0.094
1915-45	1930	30	51.3	52.4	0.053
1925-35	1930	10	(52.4)	(41.8)	(0.007)
1925-45	1935	20	57.3	27.2	-0.077
1935-45	1940	10	58.6	23.6	-0.098

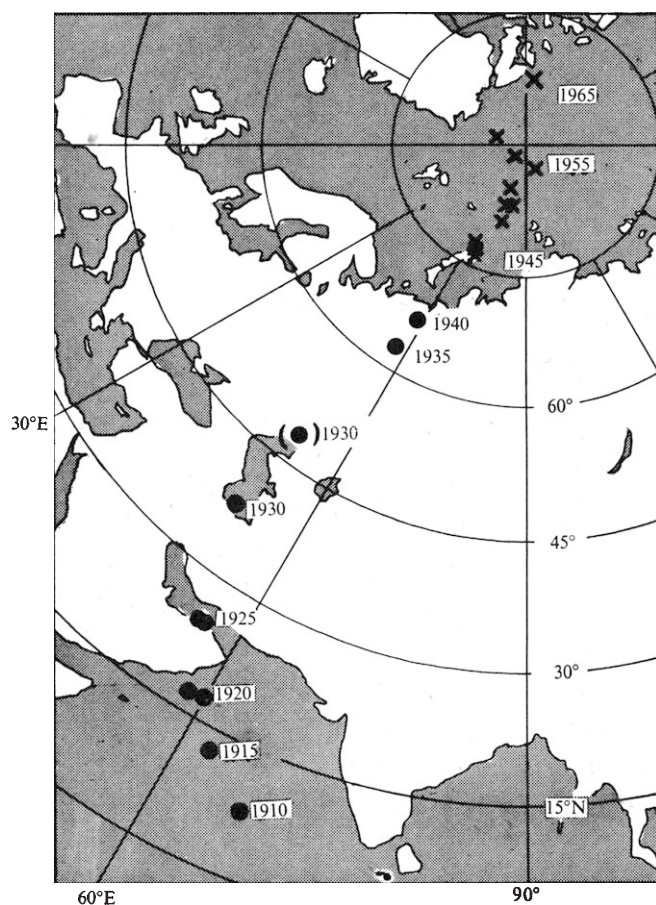


FIG. 1 Position of the optimum pole of rotation of the non-dipole part of the geomagnetic field.

between these epochs is very small and therefore the pole position is poorly determined (for a zero rotation the pole position would be indeterminate). In fact, the 1925-1935 correlation coefficient shows several maxima, and the position quoted represents only a local maximum.

The pole positions are plotted as circles on a zenithal equidistant chart (Fig. 1), together with the pole positions (indicated by crosses) obtained previously³. The results are in excellent accord with the prediction based on our earlier results. The second prediction, that the rotation rate should be smaller for earlier epochs, is also confirmed, though rather more spectacularly than we might have expected, because the rotation rate apparently drops to zero near 1930, and for earlier epochs shows the opposite sign. This is illustrated in Fig. 2, again circles and crosses denote present and previous results respectively.

Thus, the present results amply confirm our conclusion that, in general, the optimum pole of rotation of the non-dipole part of the geomagnetic field differs significantly from the geographical pole.

We have also suggested a physical interpretation of the phenomenon, based explicitly on the assumption that the rotation of the field reflected similar movements of the outer layers of the core. There are two separate parts to this assumption: (i) that the field rotates as a whole; (ii) that this rotation corresponds to a similar rotation of the outer core. It is the first of these parts that has been challenged by Lowes.

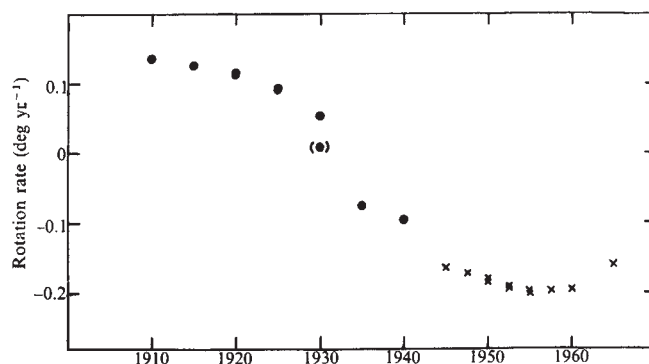


FIG. 2 Rate of rotation of the non-dipole part of the geomagnetic field about the optimum pole.

Lowes uses 'field' to denote the magnetic intensity, whereas, following Chapman and Bartels⁴, we allow it a more general meaning. Also, for 'rotation of the field', we should have written 'rotation of inhomogeneities of the field' (F. J. Müller, private communication), since rotation of an axis-symmetric field about its axis of symmetry would produce no secular change and therefore would not be detected in our investigation. For convenience, however, we retain the shorter notation.

If the whole of the secular change could be fitted by a simple rotation of the main field there would be no problem, since all the features of the field (potential, components of intensity, and so on) would yield the same pole position and rate of rotation for whatever height or depth they were analysed. It is clear, however, that such a simple model cannot account for all the complex changes in the field pattern. In the presence of such non-rotational components the derived pole position and rate of rotation will depend on the relative weights given to the individual spherical harmonic coefficients. [Two extreme examples are the potential at the surface, considered here, where the weighting is $(2n+1)^{-1/2}$ (to convert from Schmidt semi-normalisation to full normalisation), and the vertical intensity at the core-

mantle interface, favoured by Lowes, where the weighting factor is $1.83^{n+2} \cdot (n+1) \cdot (2n+1)^{-1/2}$.]

Lowes asserts that the most appropriate rotation to investigate in connection with motions of the outer layers of the core is that of the vertical intensity at the core/mantle interface, but goes on to show that, because of the high weight given to the poorly determined high-degree coefficients relative to the better determined low-degree coefficients, the resulting pole positions would be of low significance. He further suggests that secular change coefficients of degree greater than six, which are too small to be determined significantly at the surface, might well contribute a major proportion of the secular change at the core/mantle interface. For these reasons, we prefer to consider the rotation of the field at the surface, where the observations are made, and where the secular change can be well represented by spherical harmonic coefficients to the sixth degree and order. Assuming, as before, that the non-dipole field is 'frozen in' to the core, the argument may be stated as follows: if the core rotates about the deduced pole position at the deduced rate then it will reproduce the observed secular change as accurately as is possible with a simple rotation.

We concede that such a simple model is inadequate to account for all of the observed changes in the non-dipole field, but it represents a better approximation (even when the additional degrees of freedom are taken into account) than westward drift. Moreover, it has the merit of making predictions about the diurnal variation of latitude which may be tested by astronomical observation³. There seems little point in pursuing a more sophisticated physical model until the quality and distribution of secular change data permits a more reliable determination of high degree spherical harmonic coefficients.

S. R. C. MALIN

*Institute of Geological Sciences,
Herstmonceux Castle,
Sussex, UK*

*Jesus College,
Cambridge, UK*

I. SAUNDERS

Received January 22, 1974.

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Electron diffraction on liquid water

X-RAY diffraction has been successfully used in determining radial distribution function in liquid water (see references in ref. 1). The few attempts²⁻⁷ to apply diffraction of a high energy electron beam to the same end in liquids in general has not led to usable diffraction patterns, particularly in the case of liquids of higher vapour pressure⁷. To overcome the difficulties arising from the strong absorption of electrons, we produced in an electron microscope a very thin layer of liquid water nearly in equilibrium with its vapour and prevented the pressure in the vicinity of the electron beam for increasing by evaporation. The liquid layer scattered the penetrating electron beam and usable diffraction patterns could be obtained.

A chamber⁸ for an electronoptical device Zeiss EF-4 was constructed for producing a water film of controlled thickness

and temperature. The film was created by wiping a drop of the liquid over a hole 2 mm in diameter in a copper plate of thickness 0.02. This was enclosed in a chamber connected to the vacuum space only by two apertures, one of diameter 70 μm for the entering primary beam and the other of diameter 200 μm for the scattered beam. Drop and film could be reproduced many times by a suitable mechanism.

In order to avoid quick disappearance of the water film, the vapour escaping through the apertures could be replaced by evaporating the water stored in the chamber.

Usually, the primary electron beam was completely absorbed immediately after the water film was produced. By slow evaporation the thickness of the film decreased and a pattern of diffuse Debye-Scherrer rings could be seen. This was photographed on a 9×12 cm Agfa-Gevaert Scientia 23D50 plate at distances of 20, 42 and 80 cm from the water film. Thus, the ranges of the scattering angle θ and the usual parameter $s = (4\pi/\lambda) \sin(\theta/2)$ could be varied. In order to avoid a predominance of scattering at very small angles, we used an s^2 rotating sector 2 mm over the photoplate. The intensity distribution $I(s)$ on the photoplate was determined by a Zeiss GIII Schnellfotometer. The exposure time was 1 to 3 s depending on the sample-to-plate distance. Short exposure times are of particular importance in scattering from solutions in which concentration changes during the experiment are to be avoided.

Figure 1 shows a diffraction photograph (a negative print) on water at 4° C. It was taken at a sample-to-photoplate distance of 42 cm using a monochromatic electron beam of energy ~ 67 keV, corresponding to a wavelength of 0.045 Å determined experimentally. The radial distribution function in the liquid was calculated by Fourier transformation of that part of the scattered intensity which could be assumed to be coherent scattering. This was determined arbitrarily in the

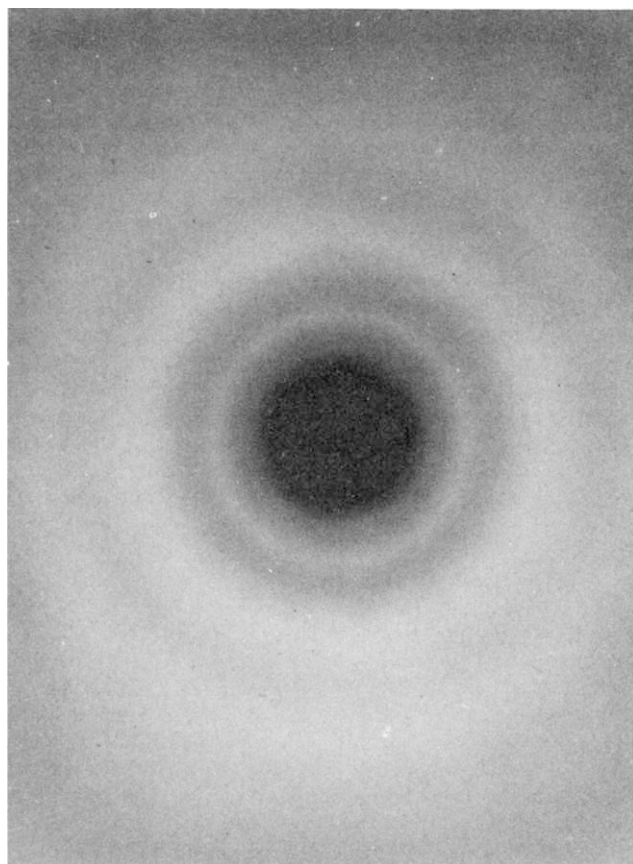


FIG. 1 Diffraction photograph (negative print) of water at 4° C.

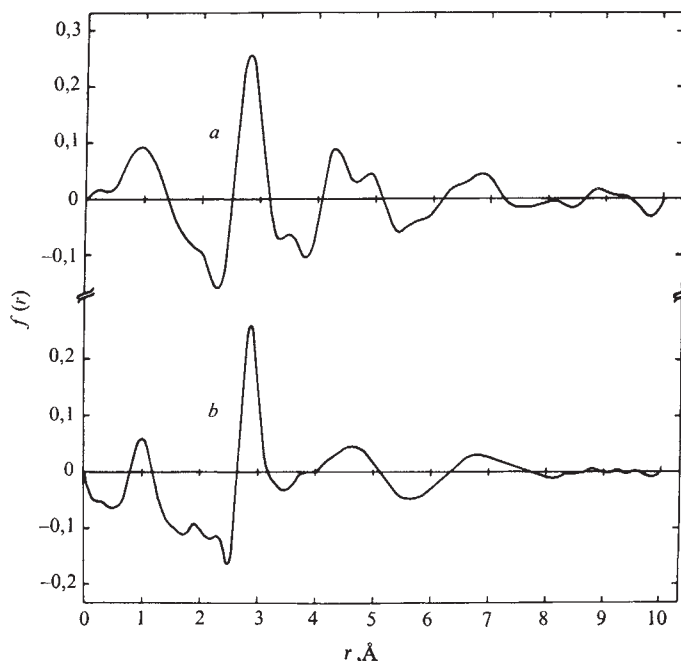


FIG. 2 Radial distribution function for liquid water, H_2O , at 4°C . a, Electron diffraction; b, X-ray diffraction^{1,9}.

following way. The modified experimental molecular intensities

$$sM(s) = s[I(s) - I_B(s)]/I_B(s)$$

were obtained by arbitrarily drawing in smooth background curves $I_B(s)$ on the total experimental molecular intensity $I(s)$ curves. The Fourier transformation

$$f(r) = \int_{s_{\min}}^{s_{\max}} sM(s) \sin(sr) ds$$

of the $sM(s)$ function was carried out in the s range 1.7 to $9.5 \text{ \AA}^{-1}$ using $\Delta s = 0.10 \text{ \AA}^{-1}$ steps.

The obtained radial distribution curve (Fig. 2a), which is not much altered by variations in the background curve, shows almost exactly the same maxima as the corresponding curves obtained from X-ray diffraction (Fig. 2b)^{1,9}.

According to the interpretation of other authors, these peaks must be ascribed, in the order of increasing r , to interactions O-H (intermolecular), O...O interactions between immediate neighbour molecules and second and more distant neighbours.

In the interaction between radiation and matter, the main difference in principle between X rays and the electron beam is that the former are diffracted by the electron clouds whereas the latter is scattered by the electric potential, depending on the steric configuration of the nuclei and the density distribution of the electrons. Consequently, electron diffraction allows the determination of the position of light atoms in the presence of heavy atoms. Therefore, in the case of water, more reliable information about the hydrogen bonds can be expected.

The energy of the electron beam generally used in diffraction experiments corresponds to a wavelength λ of some hundredths of an Ångström. This is at least ten times less than the wavelength of the X rays used for structural studies. Thus, the s range used in Fourier transformation for obtaining radial distribution from coherent intensity is much greater for electrons and so the cutoff error is reduced by comparison with X rays.

The ratio of the intensity of coherently scattered radiation to that of incident radiation is a few orders of magnitude higher in the case of electrons than in the case of X rays.

This leads to the practical conclusion that although an exposure time of a few seconds is sufficient for electrons, several hours are necessary for X rays.

SÁNDOR LENGYEL
ERIKA KÁLMÁN

Central Research Institute for Chemistry,
Hungarian Academy of Sciences,
H-1025 Budapest

Received October 8, 1973.

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BIOLOGICAL SCIENCES

Method for relating the structure and properties of chemical compounds

THE structure diagrams of chemical compounds are widely used in the communication of chemistry. They are also used in chemical information systems, mainly as keys for the retrieval of associated information¹. However some chemical information systems hold property data as well as structure diagrams in machine-readable forms²⁻⁵. If structure-property relationships could be investigated easily within chemical information systems, then the usefulness of the systems would be considerably increased. Described below are some results which have been obtained during investigation of structure-property relationships using a combination of regression analysis and some techniques of chemical structure handling used in information systems.

The method used is empirical⁶⁻⁸. The structures were coded manually as redundant connection tables and put in to the computer with the properties of the compounds. The rest of the processing was carried out automatically. A structural fragment was generated on each atom in each structure. Each fragment consisted of the central atom, the bonds it forms and the atoms to which it is bonded (excluding hydrogen atoms). Fragments of this type have been investigated⁹⁻¹⁰ and used as screens (M. F. Lynch *et al.*, personal communication, and ref. 11) in substructure search systems and for the automatic classification of chemical structures¹².

It was assumed that the value of the property under investigation, y , of the i th compound is related to its structure by the equation

$$y_i = \sum_{j=1}^n b_j x_{ij}$$

where there are n types of structural fragment in the set of structures and x_{ij} is the number of times that the j th fragment occurs in the i th structure. The constants b_j were determined by carrying out a regression analysis using a computer manufacturer's statistical analysis programs¹³.

The method was tested with a group of 79 penicillins and using the percentage serum binding as the property parameter¹⁴. They were chosen because they form a large group of structures for which property data have been published.

These compounds have also been studied using a semi-empirical structure-property correlation method and the results are available for comparison¹⁴.

An automatic analysis of the 79 penicillins in terms of the structural fragments described above showed that they contain 52 different fragment types. Eight of these however were derived from the penicillin nucleus and always occurred the same number of times in a structure. They were therefore omitted from the analysis. Also one group of four fragments and three groups of two fragments had within group correlation coefficients of 1 and thus only one fragment from each group was included. The regression analysis also excluded variables which were not significant at the 10% level.

The results of the regression analysis¹³ are shown in Table 1. The multiple correlation coefficient is 0.993 with 66 degrees of freedom ($F = 390$, $F_{12, 60, 0.001} = 3.32$) and the residual error 9.73. A regression carried out using log (amount of penicillin bound to serum/amount free) as the independent variable¹⁴ gave a multiple correlation coefficient of 0.945 with 70 degrees of freedom and a residual error of 0.294. It will be seen in Table 1 that all of the fragments except HO-C have regression coefficients which are different from zero at a significance level of 5%. The fragments which have negative regression coefficients and thus reduce serum binding are hydrophilic whereas the fragments with positive regression coefficients, which increase serum binding, are hydrophobic. The relationship between the hydrophobic nature of the side chain and serum binding of penicillins has been noted by other authors^{14, 15}.

This method differs from others published in that both the side chain and nucleus are treated in the same manner and both are broken down into fragments automatically¹⁶. It would be possible to integrate this kind of analysis easily into a computerised chemical information system. As structural fragments are developed from structure diagrams the procedure will not resolve some isomers. This situation may be improved by using larger structural fragments, or more pre-

cise forms of structural representation than the connection table used in this work. The method is subject to the usual restrictions connected with the use of multiple regression analysis. We hope to publish a more detailed account of this and related work in the near future.

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GEORGE W. ADAMSON

JUDITH A. BUSH

Postgraduate School of Librarianship and Information Science
Sheffield University
Western Bank
Sheffield, S10 2TN

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TABLE 1 Regression analysis of structural fragments

Fragment type	Regression coefficient	Student <i>t</i> statistic (66 d.f.)	Perfectly correlated fragments
NH ₂ - S	-30.64	2.23	$\left\{ \begin{array}{c} \text{O} = \text{S} \\ \\ \text{NH}_2 - \text{S} - \text{C} \\ \\ \text{O} \end{array} \right.$
Br - C	27.50	4.55	$\left\{ \begin{array}{c} \text{S} \\ \\ \text{C}^* - \text{C}^* - \text{C} \end{array} \right.$
C* S* C	17.72	3.57	$\left\{ \begin{array}{c} \text{C} \\ \\ \text{S}^* - \text{C}^* - \text{C} \\ \\ \text{F} \\ \\ \text{C}^* - \text{C}^* - \text{C} \end{array} \right.$
F - C	15.81	4.32	
NH ₂ - C	-19.69	6.18	
Cl - C	20.21	13.04	
C - CH ₂ - C	10.88	8.53	
C - O - C	6.97	2.76	
HO - C	-11.65	1.89	
CH ₃ - C	8.17	3.92	
O = C	-27.47	2.68	
C* CH* C	9.64	14.77	C - NH - C
Regression constant	91.04	2.74	

Results of the regression analysis using the serum binding percentage as the dependent variable and the occurrence of structural fragments as independent variables. The perfectly correlated fragments have correlation coefficients of +1 with the fragments in the left hand column and were not included in the calculation. All bonds are in chains except those denoted by asterisks which are delocalised ring bonds.

Terminal riboadenylate transferase in human lymphocytes

TERMINAL riboadenylate transferase (TrT) catalyses the transfer of adenylate residues from ATP to the 3'-hydroxyl group of certain polyribonucleotides in the presence of Mn²⁺. Enzymes of this kind may be involved in the processing of heterogeneous nuclear RNA to mRNA in eukaryotic cells, since many types of mRNA have terminal poly(A) sequences. Evidence for this is rather fragmentary and is complicated by reports of several forms of poly(A)-polymerising activities in both nucleus and cytoplasm¹⁻¹⁰. Clearer evidence might be obtained from systems that could be experimentally manipulated to show variation in the levels of activity of poly(A) polymerases and rates of mRNA synthesis. We have found that human lymphocytes exhibit increased TrT activity as part of their response to stimulation by phytohaemagglutinin (PHA) *in vitro*. This is the first mammalian, non-viral system in which increases in TrT activity have been observed with changes in the physiological state of the cell. The PHA-stimulated lymphocyte may be suitable for combined polymerase-mRNA turnover studies designed to clarify enzymatic steps in mRNA processing and terminal poly(A)

TABLE 1 Stimulation of polymerase activities in lymphocytes cultured with phytohaemagglutinin

Lymphocytes	Terminal ribonucleic acid transferase		Nucleus		Maxipolymerase		Minipolymerase	
	Bulk	Gradient	Bulk	Gradient	Bulk		Bulk	
Unstimulated (48 h)	28	24	0	0	1.3		0.14	
	33	15	0	0	1.2			
	21	21	0	0	1.3		0.14	
PHA-stimulated (48 h)	81	80	13	0	7.8		0.76	
	84	94	9	0	8.1		0.24	
	—	127	0	0	5.6		0.54	
	69	60	—	—	5.7		0.23	
	119	—	6	6	7.7		0.40	

Lymphocytes were cultured in RPMI-1640 tissue culture medium (Gibco) supplemented with 20% foetal calf serum, penicillin and streptomycin. Stimulated cultures (a) contained 0.2 ml Difco PHA-P stock PHA per 250 ml and controls (b) received no PHA. After the appropriate time, lymphocytes were suspended to a cell density of 1×10^6 cells ml^{-1} in TKM-sucrose buffer containing 50 mM Tris-HCl pH 7.6, 25 mM KCl, 5 mM MgCl_2 , and 0.25 M sucrose. The cells were counted, homogenised and fractionated by differential centrifugation¹⁷. The crude fractions were assayed for several enzymes directly and after velocity sedimentation through a 5–20% sucrose density gradient¹⁷. Assays for maxi and mini DNA-dependent DNA polymerases were as previously described¹⁷. The bulk assays have a total volume of 250 μl and the gradient assays were in 60 μl . The assays for TrT activity contained 0.2 M Tris-HCl, pH 8.25, 0.01 M $\text{rA}(\text{pA})_{16}$, 0.5 mM $^3\text{H-rATP}$ (3.9 c.p.m. pmol^{-1}), 0.5 mM MnCl_2 , 4mM 2-mercaptoethanol, 0.1 mg ml^{-1} bovine serum albumin and enzyme. Reaction mixtures were incubated at 35° C. For the bulk assay aliquots were removed from the reaction mixture at various times, spotted on GF/C filters, and processed for acid-insoluble activity¹⁷. Enzyme-specific activity is expressed as nmol nucleotide incorporated per 10^8 cells h^{-1} . Each line in the table represents a separate experiment.

addition, since Rosenfeld *et al.*¹¹ have reported increases in poly(A)-rich mRNA during lymphocyte transformation by PHA.

For our studies, normal human lymphocytes were separated on Hypaque-Ficoll gradients¹² and incubated with PHA (Table 1). Stimulation was monitored morphologically and there was routinely 60% blast transformation after 48 h. Thymidine pulses indicated maximal DNA synthesis at 72 h and the number of cells had approximately doubled by 96 h. For enzyme analysis cultured lymphocytes were collected by centrifugation, washed in TKM sucrose, disrupted by homogenisation, and separated into nuclear and soluble cytoplasmic fractions by differential centrifugation. In some experiments, nuclear and cytoplasmic extracts were layered on sucrose density gradients for velocity gradient fractionation. TrT and several DNA polymerase activities were assayed (Table 1.) The oligonucleotide normally used to assay TrT, $\text{rA}(\text{pA})_{16}$, was prepared by controlled hydrolysis of poly(A)¹³.

Increases in polymerase activities following treatment of lymphocytes with PHA are shown in Table 1. A typical velocity gradient separation of polymerase activities from 48 h stimulated lymphocytes is presented in Fig. 1. Direct assay of nuclear and cytoplasmic extracts (bulk assays) and

summation of gradient activities showed that most of the total TrT activity was recovered from the gradient in a single peak with an estimated molecular weight of 60,000. The stimulation of the cytoplasmic high molecular weight or 'maxi' DNA polymerase confirms the work of other investigators and will not be discussed further^{14,15}. The activity of the low molecular weight or 'mini' DNA polymerase found in the nucleus may also increase, but its activity was always much lower than that of the cytoplasmic DNA polymerase (Table 1).

The time courses of TrT, maxipolymerase and thymidine incorporation during PHA stimulation are illustrated in Fig. 2. In general TrT activity increases three to six-fold, reaching a maximum after about 48 h. Since the cytoplasmic TrT

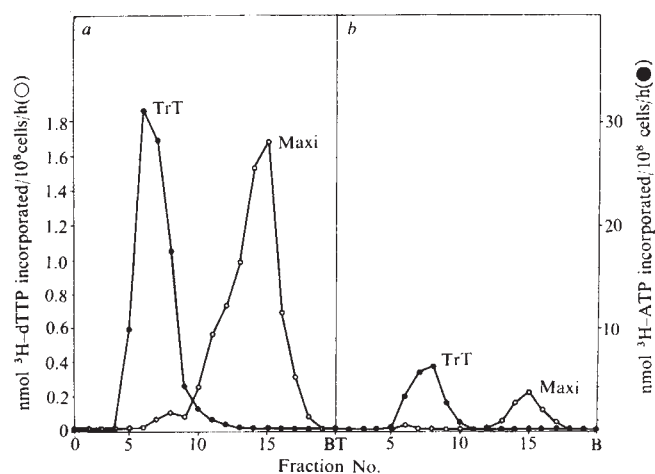


FIG. 1 Velocity gradient fractionation of polymerase activities. The soluble, cytoplasmic supernatant fractions (0.25 ml) prepared as described¹⁷ were layered on 5–20% sucrose density gradients in 50 mM Tris-HCl pH 7.6, 100 mM KCl, 1 mM 2-mercaptoethanol and 0.1 mg ml^{-1} bovine serum albumin. The gradients were centrifuged for 16 h at 40,000 r.p.m. (100,000g) and then collected in 20 fractions. Enzymes were assayed as described in Table 1. a, Stimulated lymphocytes; b, normal lymphocytes.

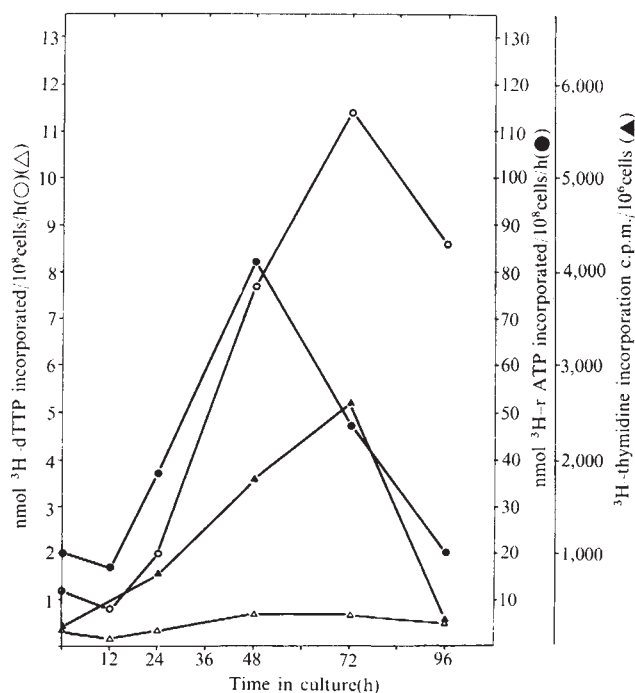


FIG. 2 Variation of polymerase activities with time after addition of PHA to cultures of human lymphocytes. For thymidine pulses, 1×10^6 cells were incubated for 2 h with 5 μCi ^3H -thymidine at 37° C. The cells were then pelleted and precipitated with cold 5% trichloroacetic acid (TCA). The TCA precipitate was collected on GF/C disks, washed, dried and counted in a liquid scintillation counter. Thymidine incorporation was linear during this time. Enzyme assays are described in Table 1. ●, Cytoplasmic TrT; ○, cytoplasmic 'maxi'; ▲, ^3H -thymidine incorporation; △, nuclear 'mini'.

assay was linear with both time and concentration of extract when a long oligoadenylate initiator was used, we feel this result is unambiguous. Cytoplasmic DNA polymerase activity and thymidine incorporation peaked at 72 h just before cell division. Most cells underwent mitosis between 72 and 96 h with a doubling in cell number. Synchronisation of cell division is good in this system. Increase in TrT activity in lymphocytes was prevented by cycloheximide addition at any time during lymphocyte activation by PHA.

The TrT activity which we detected was a soluble enzyme in the cytoplasmic fraction, was Mn^{2+} -dependent, required an oligonucleotide initiator and had a molecular weight of approximately 60,000. In crude extracts we found that an initiator 12–16 nucleotides long was optimal for detecting this activity. A low level of activity was found in the nucleus, but this result is difficult to interpret, since the enzyme could have leaked from the nucleus or bound adventitiously to chromatin upon cell breakage.

Table 2 illustrates the effects of several chemical compounds on TrT activity. Rifampicin-SV, α -amanitin and actinomycin D are all inhibitors of mammalian DNA-dependent RNA polymerases. These drugs have no effect

TABLE 2 Effect of chemicals on terminal riboadenylate transferase activity

Compound added	Percent inhibition
α -Amanitin	
8.5×10^{-6} M	1.2
1.4×10^{-5} M	0
Rifampicin	
96 μ g ml ⁻¹	0
160 μ g ml ⁻¹	0
Proflavin sulfate	
10 μ g ml ⁻¹	17
100 μ g ml ⁻¹	98
Actinomycin D	
20 μ g ml ⁻¹	3
50 μ g ml ⁻¹	2
N-Ethylmaleimide (NEM)	
0.8 mM	82
4 mM	99

TrT assays were carried out as described in Table 1. All compounds except NEM were added to the reaction mixtures. For experiments using NEM, the supernatant fraction was incubated in ice for 10 min with NEM before the addition of reaction mixture.

on TrT activity at concentrations which would inhibit RNA polymerase. Proflavin sulphate at a concentration of 100 μ g ml⁻¹ inhibits TrT. This compound also inhibits the purified TrT from calf thymus (F.J.B. unpublished data) and a similar activity in vaccinia cores¹⁸. Human TrT is a sulphhydryl enzyme, since it is completely inhibited by 4 mM N-ethylmaleimide (NEM).

These findings indicate that TrT is an enzyme distinct from DNA-dependent RNA polymerases. Its activity increases three to six-fold during PHA stimulation of the human lymphocyte. The role it plays in RNA processing and the addition of poly(A) sequences to the 3'-terminus of mRNAs may be studied more rigorously in this system which exhibits biological variation.

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MARY SUE COLEMAN
JOHN J. HUTTON
F. J. BOLLUM

Departments of Medicine and Biochemistry,
University of Kentucky Medical School,
Lexington, Kentucky 40506

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Is terminal deoxynucleotidyl transferase a somatic mutagen in lymphocytes?

AN enzyme able to add deoxyribonucleotides to the ends of DNA primers has been identified in calf thymus by Bollum and his colleagues and named terminal deoxynucleotidyl transferase^{1–4}. Chang⁵ has indicated that the enzyme is a specialised constituent of thymus cells and is not present in any other organ of several animal species. The enzyme is located in the lymphocyte fraction of the thymus (Silverstone, Parkman, McCaffrey and Baltimore, unpublished results). Recently, we found an enzyme with the properties of a terminal transferase in circulating lymphoblasts of patients with acute lymphoblastic leukaemia (ref. 6 and McCaffrey, Harrison, and Baltimore, unpublished results).

It seemed to me possible that terminal transferase could act as a mutagen, diversifying those molecules of the T lymphocyte which carry the immunological specificity of the cell. Although I realise that the question of which molecules give specificity to thymus-derived lymphocytes is open^{7,8}, I have assumed that the molecules are probably similar, if not identical, to antibody molecules. The hypothesis I wish to discuss is therefore that terminal transferase acts as a somatic mutagen to diversify the amino acid sequence in the variable region of immunoglobulin molecules. There are various hypotheses as to how the variety of amino acid sequences arises in the V regions of immunoglobulins⁹. I have assumed that somatic mutation plays a significant role: if all antibody synthesis is directly specified by inherited V regions^{9,10}, my proposal is clearly meaningless.

Numerous models of immunoglobulin diversification^{11–17}, which originate in the writing of Lederberg¹⁸ and Burnet¹⁹ postulate that most V regions arise by a process of somatic mutation onto which is superimposed a selective pressure. Starting from these models, I wish to suggest, as did Brenner and Milstein¹¹, that the mutations arise because single-stranded gaps are made in an inherited gene which encodes the V regions of an immunoglobulin, and that during the repair of these gaps, an enzyme inserts the wrong base (or wrong bases), causing a mutation. Terminal transferase

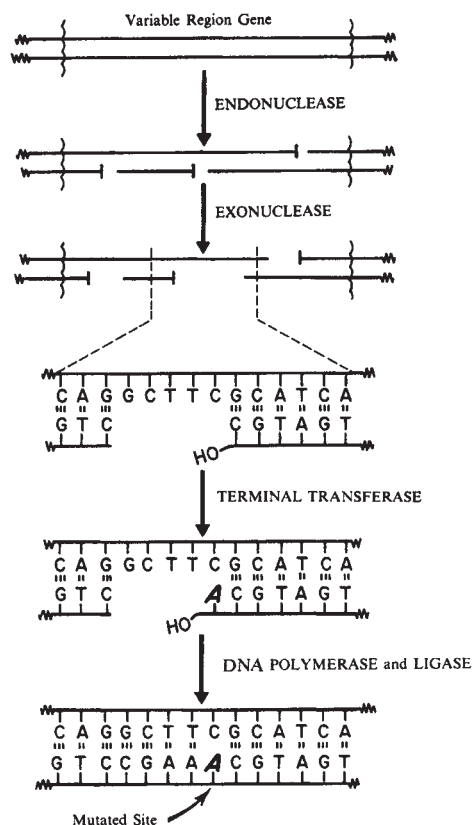


FIG. 1 Schematic representation of hypothetical mutagenic activity of terminal transferase in concert with other enzymes.

would be the mutator and thus would be the somatic 'generator of diversity'¹².

The biochemistry of terminal transferase is consistent with this model. The enzyme will add mononucleotides sequentially to any 3'-OH-terminated segment of DNA and will add the nucleotides independent of any template direction³. Ordinarily, free single strands are used as primers but the enzyme can act at gaps in double-stranded molecules almost as effectively as it does on single-stranded primers (ref. 20 and Panet, McCaffrey, Smoler and Baltimore, unpublished results).

Because terminal transferase acts non-specifically at 3'-OH ends, it must be directed to the appropriate V region in order to act on that gene but not on others. One possibility is that immune lymphocyte precursors contain a site-specific endonuclease which produces, possibly in concert with an exonuclease, the gaps at which terminal transferase can act.

A specific form of this model is presented schematically in Fig. 1. I postulate that an endonuclease makes several single-strand scissions in the V region gene of an immunoglobulin chain. Three sites of endonuclease cleavage in the V region gene are suggested because of the data from Wu and Kabat²¹ showing three hot spots of variability in the V region (hypervariable regions). The nicks are then elongated to variable length gaps by an exonuclease. The repair of one gap is shown in detail (Fig. 1). Initially, terminal transferase inserts a single, random nucleotide which, in this case, does not form a base pair with the nucleotide on the opposite strand and is therefore a mutated site. The gap is then filled by an ordinary DNA polymerase and sealed by a ligase. The filling of a gap containing a mismatched base pair at the 3'-OH end can be catalysed by a mammalian DNA polymerase²².

I postulate that a process such as that depicted in Fig. 1 causes mutations at the hypervariable positions in the V region genes^{21,23}. There are extensive differences in amino

acid sequence between V regions which are located outside the hypervariable regions. Such variation is probably due to multiple, inherited genes and not to somatic mutation²⁴⁻²⁶. These inherited differences include differences between species, differences between the subclasses of variable regions within the antibodies of a given species and differences due to allelic genetic polymorphisms in a single gene. It is significant that when data on one class of antibodies in a single inbred strain—the lambda chains of Balb/c mice—were collected^{27,28}, all the amino acid substitutions were in the hypervariable regions. In kappa chains of Balb/c mice, however, extensive variation occurs throughout the V region, suggesting the existence of many inherited V region genes²⁴. Even in the kappa chains, however, when proteins were analysed which were virtually identical outside the hypervariable regions, variability localised to the hypervariable regions was evident²⁶.

All this speculation relates to the production of circulating antibodies, but terminal transferase is found in thymocytes and it is not clear whether such cells have antibody on their surface. Some class of molecules resembling antibody must be involved in T cell specificity, however, and whatever those molecules, it seems reasonable that some of their variability arises in a manner similar to that of antibody variability.

To try to relate this model to antibody synthesis by B lymphocytes and plasma cells, we have sought terminal transferase in cells from the chicken's bursa of Fabricius. In agreement with previous results⁵, no terminal transferase like that in the thymus was evident in bursal cells (McCaffrey, Smoler and Baltimore, unpublished results). A high concentration of a unique replicative DNA polymerase which might make mistakes is, however, found in bursal cells (McCaffrey, Smoler and Baltimore, unpublished results). Immunoglobulin synthesis in B cells might therefore also involve a process of somatic diversification analogous to that described here for thymus cells. There could, however, be significant differences between the mechanisms of T and B cell diversification.

The molecular model suggested here is much more detailed than is justified by existing data. It is presented merely to show the context in which terminal transferase might act as a mutagen to diversify immunoglobulin structure. The only elements I wish to stress are the potential ability of the enzyme or enzymes like it to produce somatic variability in an inherited gene and the occurrence of such an enzyme in thymic lymphocytes. Terminal transferase could, for example, be involved in the type of somatic diversification discussed by Cunningham²⁹.

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DAVID BALTIMORE

Department of Biology,
Massachusetts Institute of Technology,
77 Massachusetts Avenue,
Cambridge, Massachusetts 02139

Received October 22, 1973; revised January 14, 1974.

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We isolated⁴ a temperature sensitive (*ts*) mutant from the Syrian hamster cell line BHK21/13, *ts422E*, which cannot grow at 39° C because at this temperature a block after formation of the 32S precursor prevents the production of 28S rRNA and 60S ribosomal subunits in the cytoplasm, while 18S rRNA and 40S ribosomal subunits are produced normally. The mutation seems to be in a gene coding for a protein involved in the processing of rRNA, the most likely possibilities being a ribosomal protein or an enzyme⁴.

The mutation seems to be recessive as it can be complemented in intraspecific hybrids with many other *ts*BHK mutants. These hybrids can all grow at 39° C⁵. In principle, the *ts422E* defect could be complemented in a hybrid cell by two different mechanisms. (a) The processing defect of *ts422E* could be relieved by the gene product of the wild type allele so that 28S rRNA transcribed from both parental cell genomes would be produced at 39° C. (b) The wild type phenotype could also be produced if synthesis of rRNA in the hybrid cells were sustained exclusively by the *ts*⁺ parental genome, while production of rRNA transcribed from the 422E genome would still be inhibited. The latter mechanism would imply that the *ts422E* mutation is not recessive, but rather partially dominant, or else it is a mutation which cannot be complemented in *trans*. We have therefore investigated how the *ts422E* mutation is complemented in hybrid cells. We took advantage of the differences in the electrophoretic mobility of the 28S rRNAs of different species^{6,7}. Since it has been shown that in mouse-hamster hybrids, the 28S rRNA of both species is synthesised⁸, we isolated hybrids between *ts422E* and mouse cells, and determined the type of 28S rRNA formed at 33° C and 39° C.

Cell culture conditions were as described before^{4,5}. *ts422E* cells were hybridised with two lines of mouse cells, C2F, a thymidine kinase deficient derivative of 3T3 cells⁹, and TG8, a hypoxanthine, guanine phosphoribosyl transferase (HGPRT) deficient derivative of 3T6 cells¹⁰. Cells were fused in the presence of β -propiolactone inactivated Sendai virus, and incubated at 33° C for 2 d. Hybrids were selected at 39° C in HAT (aminopterin 10⁻⁵ M, thymidine 4×10⁻⁵ M, hypoxanthine 10⁻⁴ M) medium¹¹. *ts422E* cannot grow at 39° C and the mouse parental cells are killed by the HAT medium. Hybrid colonies appeared with a frequency of about 10⁻⁴. Colonies were isolated and propagated at 39° C in HAT medium for two passages before transfer to 37° C in non-selective medium. Their chromosomal constitution was determined as described¹². 28S rRNA was analysed in six hybrid clones, four from the C2F cross and two from the TG8 cross, selected on the basis of their chromosome complement, that varied from a majority of hamster chromosomes

Complementation of a defect in the production of ribosomal RNA in somatic cell hybrids

THE use of cell hybridisation for complementation tests is hampered because the establishment of a hybrid cell line may involve rearrangement of both parental cells' genetic material, and often loss of chromosomes¹⁻³. Since it is difficult to recognise single chromosomes, the genotype of the hybrid cannot be determined easily. Furthermore, strong selective pressures are often applied to isolate hybrid cells. Thus, the appearance of the wild type phenotype in a hybrid between a mutant and a wild type cell does not always imply that the mutation is recessive and can be complemented by the wild type allele: alternative hypotheses must be considered.

TABLE 1 Production of hamster and mouse 28S rRNA in hybrids between *ts422E* BHK and mouse cells

Cells	Modal chromosome No.		Total production of 28S rRNA†	39° C		Total production of 28S rRNA†	33° C	
	Telocentric + acrocentric	Bi-armed		% Mouse	% Hamster‡		% Mouse	% Hamster‡
Parents								
<i>ts422E</i> (BHK)	9	30	<0.05	—	—	1	0	100
C2F (3T3)	68	0	1	100	0	1	100	0
TG8 (3T6)	90	0	1	100	0	1	100	0
Hybrids								
C12	70	58	1	15	85	1	10	90
C16	63	27	1	50	50	1	51	49
T10*	54	29	1	80	20	1	81	19
T2	82	48	1	41	59	1	43	57
C1	63	33	1	35	65	1	33	67
C7	38	56	1	26	74	1	29	71

* This hybrid had a bimodal chromosomes distribution, with about one-third of the mitotic figures having a modal number of 85 telocentric and 43 bi-armed.

† In arbitrary units, 1 corresponds to the ratio radioactivity in 28S rRNA/radioactivity in 18S rRNA obtained in wild type cells labelled with ³H-uridine for 2 h at 39° C or 3 h at 33° C, as described in Fig. 1.

‡ Calculated as described in Fig. 1

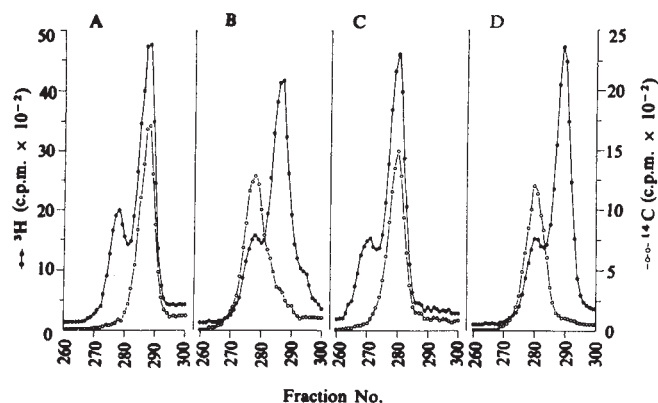


FIG. 1 Polyacrylamide gel electrophoresis of 28S rRNA from hybrid C7. Cells were shifted to 39° C or 33° C and after 2 d pulsed with ^3H -uridine ($4 \mu\text{Ci}$, $0.04 \mu\text{g ml}^{-1}$) for 2 h at 39° C and 3 h at 33° C. Cells were then washed twice with cold, isotonic Tris-buffered saline (pH 7.4) and the cytoplasmic fraction was prepared as previously described⁴. RNA was purified by two phenol extractions at room temperature and precipitated with two volumes of 95% ethanol, at -20° C. RNA was layered on linear 15-30% sucrose gradients (34 ml) in SDS buffer (0.1 M NaCl-0.5% SDS-0.01 M Tris-HCl, pH 7.2) and centrifuged at 24,000 r.p.m. for 16 h at 23° C in a SW 27 Spinco rotor. For each sucrose gradient, 1 ml fractions were collected from the bottom of the tube and the radioactivity of an aliquot was counted. The 28S rRNA peak was pooled and ethanol precipitated. 28S rRNA was fractionated by polyacrylamide gel electrophoresis: gels contained 2.6% (w/v) acrylamide and 0.13% (w/v) N, N'-methylene bisacrylamide, and were polymerised in a buffer containing 20 mM Na acetate, 1.0 mM NaEDTA, 40 mM Tris-acetate, pH 7.2, 0.2% SDS, 10% glycerol. Electrophoresis was run at room temperature, at 5 mA per gel, for 48 h in a buffer containing Tris, pH 7.2, Na acetate, NaEDTA and SDS in the same concentrations as in the gel. The desired portion of the gel was sliced frozen into 1 mm slices, solubilised in 0.5 ml of an NCS-water mixture (9:1) at 60° C overnight, and counted in a toluene-based scintillation fluid. Each 28S rRNA sample was run together with a ^{14}C -labelled marker of 28S rRNA prepared from 3T3 mouse cells or BHK cells. A, 28S rRNA (^3H) from hybrid C7 growing at 33° C (●), hamster 28S ^{14}C rRNA (○); B, 28S rRNA (^3H) from hybrid C7 growing at 33° C (●), mouse 28S ^{14}C -rRNA (○); C, 28S rRNA (^3H) from hybrid C7 growing at 39° C (●), hamster 28S ^{14}C rRNA (○); D, 28S rRNA (^3H) from hybrid C7 growing at 39° C (●), mouse 28S ^{14}C rRNA (○). Electrophoresis is from left to right. The percentage of mouse and hamster 28S rRNA produced by the hybrid was estimated by calculating the areas under each curve, which were assumed to be symmetrical.

to a majority of mouse chromosomes. Since the mouse parental lines are free of bi-armed chromosomes, the chromosome contribution of each species to the hybrids can be determined by assuming that the ratio of bi-armed to telocentric of the parental hamster cells remains unchanged (Table 2).

For the preparation of 28S rRNA, cells were labelled with ^3H -uridine for 2 h at 39° C and 3 h at 33° C (Fig. 1), to ensure that the appearance of 28S rRNA in the hybrids at

39° C would not be due to the small amount of 28S rRNA produced in *ts422E* cells at this temperature; this is generally not detectable with pulses of up to 2 h⁴. A normal amount of 28S rRNA was produced in all hybrids at both temperatures.

On polyacrylamide gels hamster 28S rRNA moves ahead of mouse 28S rRNA⁸. The 28S rRNA from each gradient was analysed on 2.6% polyacrylamide gels, 35 cm long, run for 48 h, together with appropriate markers. The separation obtainable in these conditions is shown in Fig. 1.

A summary of the results obtained with all hybrid clones examined is shown in Table 1. Hamster 28S rRNA was synthesised in all hybrids at both temperatures. The amount of hamster 28S rRNA in the hybrids varies from 20% to 90% of the total 28S rRNA, but in each hybrid was the same at 33° C and 39° C. Thus, the mutation of *ts422E* cells can be complemented by the wild type allele provided by the mouse genome.

To show that this was not due to extensive chromosomal rearrangements or selection of hybrid lines with specific chromosome losses, we fused TGS and 422E cells in the presence of Sendai virus. After 2 d of cocultivation at 33° C, the cells were plated in HAT medium at the same temperature. After 3 d, they were shifted to 39° C and kept one more day in HAT medium. After a further 24 h, they were labelled for 2 h with ^3H -uridine, and the 28S rRNA produced was analysed on polyacrylamide gels. This killed most of the mouse cells, which could not grow in HAT medium, while *ts422E* and hybrid cells were unaffected. In this population, production of 28S rRNA can be ascribed chiefly to surviving mouse cells, or mouse-*ts422E* hybrids. Accordingly, the results showed that total production of 28S rRNA was very low and, most important, 16% of this RNA was of hamster origin. In cells treated similarly in the absence of Sendai virus, hamster RNA constituted less than 4% of the total 28S rRNA. Thus, the defect in ribosome production of *ts422E* can be relieved before extensive chromosomal rearrangements in the hybrid cell, as it can be demonstrated as early as 5 d after hybridisation, corresponding to probably less than three generation times.

These findings also prove that the *ts* mutation leading to defective processing of *ts422E* rRNA does not reside in the rRNA itself, for if it did, this RNA would not be susceptible to proper processing even in hybrid cells. It is also interesting that, with respect to the protein(s) involved in rRNA processing, there can be an exchange of products between cells of different species, as mouse gene-product(s) seem able to function in the processing of hamster rRNA.

It has been reported that in mouse-hamster hybrids, when the chromosomes of one species constituted most of the chromosomes of the hybrid, a disproportionately higher percentage of rRNA of that species was present⁸. We do not find this relationship in all our hybrids (Table 2), and at least two (T2 and C16) produce approximately equal amounts of mouse and hamster 28S rRNA. It does not seem likely that this discrepancy is due to different methods of labelling the cells (2-3 h compared with steady state label in ref. 8)

TABLE 2 Chromosomal constitution and production of hamster and mouse rRNA of *ts422E* × mouse cell hybrids

Hybrids	Modal chromosome No.			Total hamster*	% Hamster chromosomes	% Hamster 28S rRNA
	Telocentric	Bi-armed	Total mouse*			
C7	38	56	22	72	76	71
C12	70	58	53	75	58	85
T2	82	48	68	62	47	57
T10	54	29	45	38	46	19
C1	63	33	53	43	45	65
C16	70	58	55	35	39	49

Production of hamster 28S rRNA was calculated as described in Fig. 1 and Table 1.

* Estimated by assuming that the number of hamster telocentrics is proportional to the number of bi-armed chromosomes.

for labelling our hybrid cells for 2 or 24 h did not substantially change the proportion of mouse and hamster 28S rRNA. This discrepancy is more likely to reside in the nature of the hybrid lines examined: we examined ours soon after formation, but Eliceiri⁸ examined hybrids which had undergone extensive propagation and recloning¹². If the production of predominantly one species of 28S rRNA conferred even a slight growth advantage to these cells, this would lead to the selection of stable hybrid lines containing predominantly rRNA of one of the two species.

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DANIELA TONIOLO
CLAUDIO BASILICO

Department of Pathology,
New York University
School of Medicine,
New York, New York 10016

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Changes in a surface-labelled galactoprotein and in glycolipid concentrations in cells transformed by a temperature-sensitive polyoma virus mutant

IN hamster cells transformed by a temperature-sensitive mutant of polyoma virus we have found that the external label in a galactoprotein disappears and the cellular concentration of lactosylceramide increases when the cells are grown at the permissive temperature. These changes can be reversed at the non-permissive temperature.

Three kinds of major membrane changes involving glycoproteins and glycolipids are associated with malignant transformation. They are (1) enhanced agglutinability of cells by some sugar-binding proteins (lectins)¹⁻³; (2) blocked synthesis of gangliosides⁴⁻¹⁰, neutral glycolipids¹¹⁻¹³, fucolipids^{14,15} and some glycoproteins¹⁶ with occasional accumulation of precursor glycans^{4,6,10}; and (3) enhanced synthesis of sialofucoglycopeptide^{17,18}. Recently a method for surface-labelling cells using galactose oxidase followed by treatment with tritiated sodium borohydride has been used to distinguish between surface galactoproteins of normal and transformed cells¹⁹. A galactoprotein which was labelled in normal hamster NIL cells was not labelled in polyoma-transformed NIL cells, and the introduction of label into this surface moiety seemed to be a correlative of growth control.

The enhanced agglutinability of transformed cells¹⁻³ has been linked directly to the control of cellular multiplication

in two types of transformed cells harbouring thermosensitive mutations. In one case, what is believed to be a mutation in a cellular gene results in the restoration of regulated growth and decreased agglutinability at the restrictive temperature, while the cells seem to be transformed at the permissive temperature^{20,21}. The same phenomenon is observed in cells transformed by thermosensitive polyoma virus mutant ts3^{22,23}.

We have initiated an investigation of the biochemical basis for the thermosensitive changes in the surface membrane of polyoma ts3-transformed BHK cells. Since topoinhibition of DNA synthesis is also rendered thermosensitive in these same cells²², we speculate that the temperature-dependent surface alterations that we have observed may be implicated in the control of DNA synthesis.

BHK cells transformed with temperature-sensitive polyoma virus (BHKpyts3 Cl 7C), those transformed with wild type polyoma virus (BHKpywt Cl 4), and their progenitor cells (BHK Cl 13) were obtained from Dr W. Eckhart at the Salk Institute. Each cell line was grown in Dulbecco's

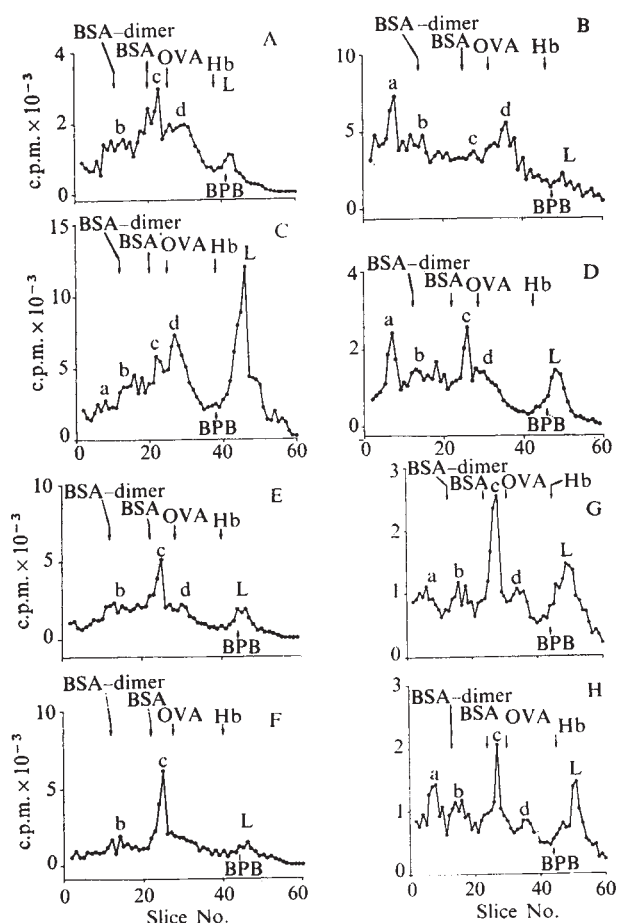


FIG. 1 Profile of external galactosyl label in baby hamster kidney fibroblasts (BHK C13) on acrylamide gel electrophoresis in sodium dodecyl sulphate. Cells were treated on the plates with 10 Worthington units of galactose oxidase for 1 h at room temperature, taken off with EDTA, and labelled as described previously¹⁹. Graphs show subconfluent non-transformed BHK at 32°C (A) and growing at 39°C (C); confluent non-transformed BHK at 32°C (B) and at 39°C (D); wild-type-polyoma-transformed BHK at confluency at 32°C (E) and at 39°C (F) ts3-transformed BHK at confluency at 32°C (G) and at 39°C (H). Acrylamide concentration was 5%; slicing, counting and preparation of internal ¹⁴C-labelled standard proteins were as previously described¹⁹. BSA, bovine serum albumin; OVA, ovalbumin; Hb, haemoglobin; BPB, bromo-phenol blue. Note that galactoprotein a is only pronounced in confluent, non-transformed cells and in BHKpyts3 at non-permissive temperature. Peaks c and d are non-specifically labelled by sodium borotritiate alone. L = lipid label.

TABLE 1 Dependence of galactoprotein a label on cell density in normal BHK cells, cells transformed with wild-type polyoma virus (BHKpywt) and with *ts3*

Cells	Temperature (°C)	Presence or absence of galactoprotein a label		
		Sparse*	Subconfluent†	Confluent‡
BHK normal	32	—	—	++
	39	—	+	++
BHKpywt	32	n.d.	—	—
	39	n.d.	—	—
BHKpyts	32	n.d.	—	—
	39	n.d.	+	++

The apparent molecular weight of galactoprotein a was approximately 200,000. —, Absent; +, obviously present; ++, present in high quantities; n.d., not determined.

* Cells growing separately without touching.

† Cells touching but not saturated.

‡ Cells at saturation density.

modified minimum essential medium containing 10% foetal calf serum at the permissive (32° C) and non-permissive (39° C) temperatures for the *ts3* gene function.

Surface labelling was achieved on cells growing on plastic dishes by the method described previously¹⁹. Glycolipids were extracted with chloroform-methanol and isolated by acetylation: the glycolipids were separated on thin-layer chromatography into fractions lactosylceramide, trihexosylceramide and haematoside. Each fraction was extracted from silica gel with chloroform-methanol-water (1:1:0.1) and the extracts were methanolysed, and the sugars liberated were determined as trimethylsilyl derivatives with myo-inositol as an internal standard by gas-liquid chromatography.

As Table 1 and Fig. 1 show, in cells grown to their maximum saturation density by changing the medium frequently, a surface galactoprotein of BHK with a molecular weight of 200,000 (galactoprotein a) was not labelled in BHKpywt either at 32° C or at 39° C, nor was it labelled in BHKpyts3 at 32° C. However, galactoprotein a of BHKpyts3 was labelled when the cells were grown at 39° C, where they acquired normal morphological appearance, decreased agglutinability with lectins and increased topoinhibition of DNA synthesis.

In actively growing, sparse or subconfluent BHK cells, galactoprotein a was not labelled, whereas it was labelled when growth was inhibited at the saturation density.

As Table 2 shows, in BHKpywt the chemical concentration of lactosylceramide was obviously higher than that of normal BHK cells, and the concentration of trihexosylceramide was considerably reduced. The concentration of lactosylceramide was also sharply increased in BHKpyts3 at 32° C but not at 39° C, whereas the concentration of haematoside was depressed. It is noteworthy, however, that the concentration of trihexosylceramide was quite low at both temperatures in BHKpyts3.

Recently, Hammarström and Bjursell²⁴ observed that isotope incorporation from ¹⁴C-palmitate into trihexosylceramide depends on cell population density and decreased

considerably when the cells were transformed with wild type polyoma virus, in agreement with our previous findings¹⁰. Also consistent with their results, the decreased synthesis of trihexosylceramide in BHKpyts3 at the permissive temperature was not restored at the non-permissive temperature, indicating that decreased synthesis of trihexosylceramide is not essential for expression of certain aspects of the transformed phenotype. Similarly, the growth behaviour in agar and the serum requirement of BHKpyts3 are not known to be temperature-sensitive²², although topoinhibition of DNA synthesis and agglutinability by lectins are temperature-sensitive^{22,23}. Thus, although trihexosylceramide reduction accompanies transformation by polyoma virus, it is not under the control of the *ts3* gene function and therefore does not seem to be implicated in the regulation of cell growth.

A considerable increase of lactosylceramide in some clonal isolates of BHKpywt had also been observed previously⁴. Our results suggest that increased lactosylceramide could be essential for expression of crucial aspects of the transformed phenotype, including the regulation of growth.

The inability to label galactoprotein a in the transformed state shows a good parallel with the appearance of lectin agglutinability, as demonstrated reversibly in BHKpyts3 at permissive and non-permissive temperatures²³. The labelling of the galactoprotein in non-transformed cells at confluency is consistent with an increased reaction of galactosyl residues with *Ricinus communis* protein as observed by Nicolson²⁵. Our surface labelling procedure did not label lactosylceramide of either transformed or non-transformed BHK cells, in agreement with the finding that the lactosyl moiety may not protrude towards the outside of the cells or that the length of the lactosyl moiety is too short to be externally labelled due to steric hindrance.

Perfect reversibility in the labelling of galactoprotein a and the lactosylceramide and haematoside concentrations in BHKpyts3 between permissive and non-permissive temperatures suggest that these membrane parameters are related

TABLE 2 Glycolipid concentration of BHK cells, those transformed with wild-type and with *ts3*

	Temperature (°C)	Glycolipid Concentration $\mu\text{mol per 100 mg dry weight}^*$		
		Ceramide† trihexoside	Ceramide† dihexoside	Haematoside§
BHK normal	32°	0.070	0.218	0.46
	39°	0.075	0.161	0.93
BHKpywt	32°	0.008	0.371	0.77
	39°	0.015	0.329	0.75
BHKpyts	32°	0.040	0.725	0.12
	39°	0.015	0.120	1.21

Concentrations were determined by gas-liquid chromatography.

* Dry weight of the cell residue.

† $\alpha\text{Gal} \rightarrow \beta\text{Gal} \rightarrow \text{Glc} \rightarrow \text{ceramide}$.

‡ $\beta\text{Gal} \rightarrow \text{Glc} \rightarrow \text{ceramide}$.

§ Sialyl 2 $\rightarrow 3\beta\text{Gal} \rightarrow \text{Glc} \rightarrow \text{ceramide}$.

to growth control in BHK cells. It is possible that deletion of galactoprotein α initiates uncontrolled DNA synthesis, as the loss of this protein has been observed after trypsin treatment of BHK and NIL cells (our unpublished observation), and since trypsin is known to induce the initiation of S-phase, and mitosis²⁶.

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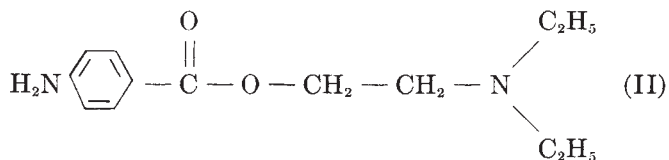
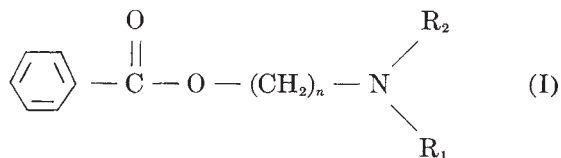
CARL G. GAHMBERG
DON KIEHN
SEN-ITIROH HAKOMORI

Departments of Pathobiology and Microbiology,
University of Washington,
Seattle, Washington 98195

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role in their anaesthetic action⁵⁻⁶. If these esters are found to be radiosensitisers they may prove very valuable in radiation therapy. Here we present data on one such compound, procaine hydrochloride (II) which is commonly used as a local anaesthetic and has been found to enhance radiation lethality of *Escherichia coli* B/r under anoxia.



E. coli B/r cells grown to stationary phase in nutrient broth (Difco) were suspended in 0.1 M phosphate buffer (pH 7) at a concentration of approximately 2×10^8 cells ml⁻¹. The cell suspension was irradiated in a ⁶⁰Co γ-ray source at a dose rate of 8 krad min⁻¹ and the radiation dose was measured by ferrous sulphate dosimetry. The cell suspension was deoxygenated by bubbling oxygen-free nitrogen before and during irradiation. Sodium nitrate (Analar) and commercially available medical grade nitrous oxide were used as electron scavengers. Procaine hydrochloride (USP) obtained from Hoechst Pharmaceuticals (India) was used without further purification. Toxicity of procaine hydrochloride towards bacteria was determined up to a contact time of 100 min in buffer and only non-toxic concentrations were used in the present studies. Irradiated samples were diluted in sterile phosphate buffer, inoculated into Petri plates and overlaid with nutrient agar (Difco) previously melted and held at 48° C. A minimum of 500 colonies of bacteria were counted at the end of 18 h incubation at 37° C. All the survival curves represent data from experiments repeated at least three times with replicate plates at all dilutions. From the exponential part of the survival curves plotted on a semi log scale, *D*₁ (dose for 1% survival) values were obtained and the dose modification factor (DMF) was calculated as the ratio of *D*₁ in the presence of procaine HCl and that of the control cells.

Procaine HCl at a concentration of 250 μM enhanced the lethality of *E. coli* B/r cells on irradiation under anoxia (DMF = 0.54). It can be seen from Table 1 that a further increase in procaine concentration did not show proportionate decrease in DMF. Whereas procaine HCl was effective also when cells were irradiated under anoxia in nutrient broth, there was no effect if cells treated with the chemical were thoroughly washed before irradiation. Procaine HCl irradiated in aqueous solution under anoxia did not show any toxic effects on unirradiated bacteria indicating that the enhancement of the radiation lethality was not due to some stable toxic products of the anaesthetic. On the other hand, when irradiated cells were immediately mixed (within 1 min of irradiation) with unirradiated procaine HCl there was a significant enhancement in the lethality (Table 1) implicating the inhibition of post-irradiation repair. This would also rule out the involvement of any short-lived transients of the chemical in this process. The fact that the effect of procaine HCl was observed even when irradiation was carried out in the presence of broth or in the presence of an efficient electron scavenger, NaNO₂, at equimolar concentrations (Table 1) further supports this view.

Enhancement of radiation lethality of *E. coli* B/r by procaine hydrochloride

MANY carbonyl compounds by virtue of the electron affinitive property¹ of their C=O groups are good radiosensitisers in bacterial as well as mammalian cells²⁻⁴. The electronic charge distribution at the carbonyl bond of some local anaesthetics belonging to the class of esters represented by the general formula (I) given below is also known to play an important

TABLE 1 Modification of radiation response of *E. coli* B/r by procaine hydrochloride

Medium	Gas	Procaine HCl mM	Other treatment	D_1 (krad)	DMF
Buffer	N ₂	—	—	74.0 ± 1.4	—
	Air	—	—	24.2 ± 1.1	0.33
	N ₂ O	—	—	68.0	0.92
	N ₂	—	NaNO ₃ (2 mM)	70.0	0.94
	N ₂	0.25	—	40.0 ± 0.6	0.54
	N ₂	0.50	—	40.0 ± 0.6	0.54
	N ₂	2.00	—	37.5 ± 1.2	0.51
	N ₂	10.00	—	36.8 ± 1.6	0.50
	N ₂	25.00	—	30.9 ± 1.0	0.42
	N ₂	25.00	Chemical washed off before irradiation	74.0	1.00
	N ₂	25.00	Chemical washed off after irradiation	31.2 ± 1.3	0.42
	N ₂	25.00	Irradiated cells mixed with unirradiated procaine HCl	38.5 ± 1.5	0.52
	N ₂	2.00	NaNO ₃ (2 mM)	38.0	0.51
	Air	25.00	—	26.0 ± 2.0	0.35
	N ₂ O	25.00	—	66.5 ± 1.5	0.90
Nutrient broth	N ₂	—	—	87.0 ± 7.0	—
	Air	—	—	28.0 ± 2.0	0.32
	N ₂	25.00	—	43.5 ± 1.5	0.50
	Air	25.00	—	28.0 ± 2.0	1.00

Whereas the actual mechanism still remains uncertain, procaine HCl was not found to react with cysteine (our unpublished results) as studied by Ellman's reaction modified by Butterworth *et al.*⁷ This could explain the result that cells treated with procaine HCl but irradiated after being thoroughly washed were not affected. The local anaesthetics and procaine HCl in particular, are known to interact with cellular membranes⁸ leading to various changes in membrane characteristics^{9,10}. Since membrane is believed to play an important role in the modification of radiation response of cells^{11,12} it is likely that the enhancement of radiation lethality by procaine HCl may be somehow related to its interaction with the bacterial membrane. The enhancement of damage by N₂O (ref. 13) (Table 1) could also be attributed to its anaesthetic action although alternative mechanisms¹⁴ have been suggested. Shortlived transients do not seem to play any significant role and so the observation that N₂O₁, which is also an electron scavenger, considerably reduced the effect of procaine HCl (DMF = 0.90) indicates a competition between the two anaesthetics for sites to interact with the cellular membrane, rather than with the radiolytic transients of water.

The ineffectiveness of procaine HCl under oxic conditions of irradiation still needs a satisfactory explanation. The following facts, however, must be kept in mind while suggesting any mechanism for this effect: (i) free radical intermediates may not be playing any role in enhancement of damage by procaine HCl; (ii) oxygen itself enhances radiation effects implicating the cellular membrane¹⁵; (iii) the radiation damage caused in presence of oxygen is generally non-repairable¹⁶.

M. A. SHENOY
B. B. SINGH
A. R. GOPAL-AYENGAR

Bio-Medical Group,
Bhabha Atomic Research Centre,
Trombay, Bombay 400 085

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Demonstration in mouse of X-ray induced deletions for a known enzyme structural locus

THE known lethal alleles at the albino locus of mice are radiation-induced and cause prenatal or early neonatal death. Four alleles expressed in the homozygous newborn as absence of pigment and early neonatal death (c^{1408} , c^{112K} , c^{65K} , and c^{3H}) were found to cause hypoglycaemia and deficiency of glucose-6-phosphatase and other enzymes¹⁻³. Two additional radiation-induced alleles (c^{6H} and c^{25H}) act as early embryonic lethals. Complementation tests between these lethal-albino alleles suggested that all six mutations could be deficiencies for genetic material other than just the albino locus¹. Since X rays are known to produce small deletions^{4,5}, all six of these mutations have been tested with various closely linked genes using the standard deletion mapping procedure. Unfortunately, the two most closely linked genes to *c*, *taupe* (*tp*) and *shaker-1* (*sh-1*) (order with centi-Morgans (cM) distance, *tp* — 2 — *c* — 4 — *sh-1*), seemed not to have been deleted in any of the six lethal-albino mutations^{2,6}. Recently,

TABLE 1 MOD-2 phenotypes of individuals produced by crossing c^{ch}/c^* to SM/J mice.

Genotype of original c^{ch}/c^* individual	^a Untyped F ₁ offspring		^b Typed F ₁ offspring			
	MOD-2AB	MOD-2A	$c^{ch}/+$ MOD-2AB	MOD-2A	$c^*/+$ MOD-2AB	MOD-2A
$c^{14CO8} Mod-2^?$ $c^{ch} Mod-2^b$	11	0	2	0	2	0
$c^{6H} Mod-2^?$ $c^{ch} Mod-2^b$	4	5	3	0	0	5
$c^{25H} Mod-2^?$ $c^{ch} Mod-2^b$	3	3	5	0	0	5

the gene mitochondrial malic enzyme (*Mod-2*) that codes for electrophoretic variants of mitochondrial malic enzyme (MOD-2: EC 1.1.1.40, L-malate: NADP oxidoreductase, decarboxylating) was shown to reside in chromosome 7 one cM from *c* between *c* and *sh-1* (ref. 7 and E. M. E. and D. L. Coleman, in preparation). We present evidence here showing that two of these mutations (c^{6H} and c^{25H}), but not the c^{14CO8} mutation, are deletions that extend into the *Mod-2* locus.

Stocks of each of the lethal-albino alleles are maintained by brother-sister mating of heterozygous individuals containing the chinchilla (c^{ch}) allele at the *c* locus and one of the lethal-albino alleles (c^*). At the time of weaning, the phenotype of homozygous chinchilla (c^{ch}/c^{ch}) is darker than that of heterozygotes for chinchilla and albino (c^{ch}/c^*). In the crosses reported here, the normal *c* allele (+) was obtained from randomly-bred Swiss-Webster mice. The *Mod-2^a* allele was obtained from the SM/J strain maintained at the Jackson Laboratory. The lethal-albino stocks and the Swiss-Webster stock carried only the *Mod-2^b* allele.

Individual hearts were homogenised in one to two volumes of distilled water and then Triton X-100 was added to give approximately a 1% final concentration. The supernatant was prepared by centrifuging the homogenate at 20,000 *g* for 15 min and applied directly to the gel wells. Electrophoresis was performed in a Buchler Vertical Starch Gel Apparatus, cathode to anode, at 150 V for 18 to 20 h. The bridge buffer consisted of 0.233 M Tris base, 0.086 M citric acid (pH 7.0). The gel consisted of 12% Electrostarch in a gel buffer of 0.0043 M Tris base, 0.0016 M citric acid (pH 7.0). The gel was sliced and stained according to the method of Shows *et al.*⁸

Individual c^{ch}/c^* mice (MOD-2B), where c^* represents either the c^{6H} , c^{25H} , or c^{14CO8} allele, were crossed to SM/J

mice (MOD-2A) and the *Mod-2* phenotype of individual offspring was determined (Table 1, Column a). In the cross involving c^{14CO8} only one type of offspring was obtained, namely that with the heterozygous (*Mod-2^a/Mod-2^b*) phenotype, MOD-2AB. But in the crosses involving either c^{6H} or c^{25H} , segregation of two types of offspring occurred: those with the heterozygous (*Mod-2^a/Mod-2^b*) phenotype, MOD-2AB, and those that displayed the MOD-2A phenotype characteristic of the SM/J strain (Table 1). This raised the suspicion that perhaps individuals carrying one of the two lethal-albino alleles, c^{6H} or c^{25H} , might lack the *Mod-2* locus in *cis* position with c^* accounting for the lack of expression of the *Mod-2^b* allele. This hypothesis was put to test by progeny testing these F₁ individuals derived from crosses of c^{ch}/c^* (MOD-2B) by $+/+$ (SM/J, MOD-2A). Crosses of these to a Swiss Webster albino stock (*c/c*), permitted the identification of the particular allele, c^{ch} or c^* , at the albino locus. Only those F₁s which carried c^{6H} or c^{25H} lacked the *Mod-2^b* allele whereas this was present in all those F₁s carrying the c^{ch} -allele (Table 1, Column b and Fig. 1).

We believe this is the first demonstration in mammals of X-ray induced deletions for an identified enzyme structural locus. The c^{6H} and c^{25H} deletions extend in length at least 1 cM, since *Mod-2* is involved. Our data are also consistent with the genetic complementation map constructed for the six lethal-albino mutations where c^{6H} and c^{25H} complement with c^{14CO8} but not with each other⁹. The data suggest that mitochondrial malic enzyme may be essential in embryonic development as the two lethal albino alleles in which its structural locus is deleted are early embryonic lethals.

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ROBERT P. ERICKSON

Department of Pediatrics,
University of California, San Francisco,
California 94143

EVA M. EICHER

The Jackson Laboratory,
Bar Harbor,
Maine 04609

SALOME GLUECKSOHN-WAELSCH

Department of Genetics,
Albert Einstein College of Medicine,
Bronx, New York 10461

Received November 26, 1973.

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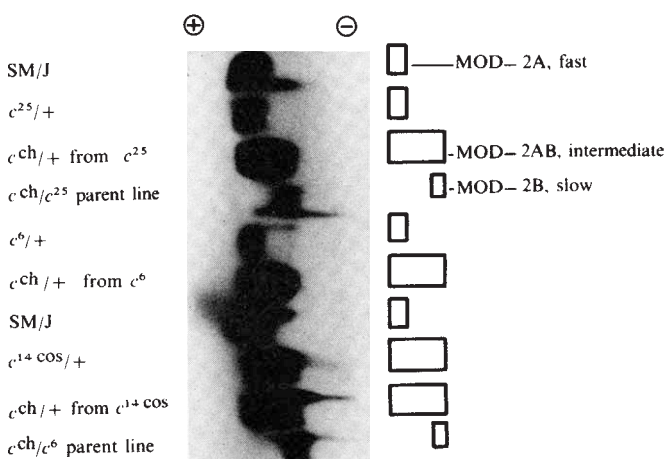


FIG. 1 The MOD-2 phenotypes of SM/J, lethal-albino stocks, and lethal-albino X SM/J F₁ mice whose genotypes were determined by a cross to Swiss-Webster mice. Heart extracts were run on starch gel electrophoresis.

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Gene dosage in a deletion for a nuclear-coded, mitochondrial enzyme

It is a general principle of mammalian biochemical genetics that enzymatic activity is proportional to gene dosage; that is, co-dominance of the biochemical phenotype is usual in heterozygotes. A well studied exception is the lethal albino locus of mice. The radiation-induced lethal albino alleles cause prenatal or early neonatal death. The mutations are unusual in their lack of gene dosage effect. Four alleles (c^{1400} , c^{112K} , c^{65K} , c^{3H}) that are expressed in newborns as absence of pigment, hypoglycaemia, and neonatal death are associated with glucose 6-phosphatase deficiency^{1,2}. Heterozygotes distinguishable by pigment dilution have the same glucose 6-phosphatase levels as normal homozygotes in a number of situations, including enzyme induction with corticoids, glucagon, dibutyryl cyclic AMP, by producing alloxan diabetes, and during normal post-natal development^{3,4}. Four other enzymes (tyrosine aminotransferase, serine dehydratase, bilirubin glucuronyl transferase, and spectrophotometrically-determined cytochrome P-450) that are deficient in lethal albino homozygotes are present in normal levels in heterozygotes (refs 3-5 and M. M. Thaler, R. P. E., and S. Gluecksohn-Waelsch, in preparation). There is evidence that mutation of something other than structural genes for the enzymes is involved. Residual amounts of glucose 6-phosphatase and tyrosine aminotransferase are present in lethal albino homozygotes. Complementation of these lethal albino alleles with two additional lethal albino alleles, c^{6H} and c^{25H} obtained from Harwell, resulted in normal enzyme levels^{4,5}. Also, abnormalities of endoplasmic reticulum of lethal albino hepatocytes are strikingly evident on electron microscopy⁶. Enzymes deficient in homozygotes are either cyclic AMP-induced or microsomal. A mutation in the gene for a membrane structural protein or in the gene for a component of the cyclic AMP induction system has been suggested as an underlying defect which might account for the deficiency of a number of enzymes and the failure to demonstrate heterozygote dosage effect, although biochemical and immunological techniques have not yet detected any abnormality (R. P. E., P. Siekevitz, K. Jacobs, and S. Gluecksohn-Waelsch, in preparation).

It has been demonstrated that in two lethal albino alleles (c^{6H} and c^{25H}) which cause early embryonic death the deletion extends to the adjacent structural locus for mitochondrial malic enzyme (*Mod-2* EC 1.1.1.40, L-malate NADP oxidoreductase, decarboxylating)⁷. We compared malic enzyme activity in hearts and kidneys of lethal albino heterozygotes, which possess one copy of the structural gene, to their normal littermates, which possess two copies. A 2:1 ratio of enzyme activity is expected if dosage holds true. Typed offspring of the c^{ch}/c^{6H} or $c^{ch}/c^{25H} \times SM/J (+/+)$ had been tested by the cross to Swiss Webster (c/c)⁷. These

typed animals were either hemizygous (*Mod-2a*) or heterozygous (*Mod-2ab*) at the structural locus for mitochondrial malic enzyme.

Adult mice were killed by cervical dislocation. All operations were carried out at 0° C. 73% of malic enzyme in mouse hearts is mitochondrial⁸. Homogenates of individual hearts were prepared by homogenising minced tissue in 0.5 ml of distilled water. Triton was added to the homogenate to make a 1% solution, which was incubated for 15 min and then centrifuged at 700g for 30 min. The supernatant was collected for enzyme assay and protein quantification.

Kidney mitochondria were purified by homogenising the kidneys from individual animals in three volumes of 0.25 M sucrose in 0.01 M, pH 8 glycylglycine. The homogenate was centrifuged at 700g for 10 min. The supernatant was collected and spun at 8,700g for 15 min. The resulting pellet was resuspended in 1.0 ml of sucrose-glycylglycine and Triton was added to make a 1% solution. After incubating for 15 min, the preparation was centrifuged at 39,000g for 20 min and the supernatant saved for assay and protein quantification.

Assay for malic enzyme activity was carried out with a spectrophotometer measuring change in optical density at 340 nm at 37° C. The total volume was 0.2 ml and final concentrations of the reagents were: glycylglycine, 0.2 M; $MnCl_2$, 0.79 mM; NADP, 2 mM; and, L-malic acid, 16.5 mM. Malic acid was added to start the reaction after the other reagents and an appropriate dilution of enzyme preparation had equilibrated at 37° C for 5 min. Protein concentrations of the enzyme preparations were determined by Lowry's method which was not affected by the low concentrations of Triton X-100 present.

As seen in Table 1, about half as much malic enzyme was found in hearts from the hemizygous mice as was found in normal, heterozygous mice. The difference is highly significant. In fact, there is a better fit to the 2:1 ratio than is expected for material where only 73% of the enzyme is mitochondrial. Given the standard errors, the activity ratio is not greatly different than the expected 63.5:100. A significant difference was however not found with the purified kidney mitochondria although the trend is in the expected direction. The difference is not due to a different specific activity of the MOD-2A enzyme (the fast form solely present in the hemizygous mice) as *Mod-2a* homozygotes (SM/J strain) showed 35.0 ± 4.4 nmol per min per mg protein in hearts and 54.6 ± 5.6 nmol per min per mg protein in purified kidney mitochondria.

We conclude that the principle of gene dosage for enzymatic activity in heterozygotes also applies for a deletion in the structural gene of mitochondrial malic enzyme. This is in contrast to the failure to observe dosage effects in lethal albino heterozygotes with glucose 6-phosphatase and a number of other enzymes that are deficient in lethal albino homozygotes. It seems likely that these exceptions to gene dosage are related to a defect in a structural component, for example, microsomal membrane protein or in regulation rather than mutation of structural genes. Thus, gene dosage in mammals cannot always be explained by normal levels of enzymatic protein of which half is nonfunctional (but which might often be detected as CRM as has been found with human gal-1-P uridyl transferase deficiency⁹).

It is interesting that gene dosage is present for a nuclear coded mitochondrial enzyme. In yeast and *Neurospora*, blockage of mitochondrial transcription and translation stimulates synthesis of nuclear coded mitochondrial enzymes, suggesting that mitochondria make a repressor(s) regulating nuclear gene expression¹⁰. The finding that the deletion of one nuclear gene for a mitochondrial enzyme leads to a halving of the enzyme's activity would suggest that activator controls, if present in mammals, are not locus specific. The detection of human, nuclear controlled, mitochondrial en-

TABLE 1 Malic enzyme activity in mice with a deletion for mitochondrial structural gene (*Mod-2*) and normals

	nmol/min/mg protein	
Tissue genotype:	$c^6/+$ or $c^{25H}/+$	$c^{ch}/+$
heart homogenate	(6) $18.0 \pm 1.5^*$ $t = 4.58, P < 0.01$	(5) 39.4 ± 3.9
kidney mitochondria	(6) 46.6 ± 4.3 $t = 1.97, P = 0.1-0.05$	(5) 59.8 ± 5.1

* (No. of animals) mean $\pm$ s. e.

zymes in putative mouse mitochondria in man-mouse somatic cell hybrids¹¹ might also suggest that such controls are not species specific. Mammalian nuclear-mitochondrial interactions may involve different control mechanisms.

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Note added in proof: E. M. Eicher and D. L. Coleman (personal communication) find dosage for MOD-2 in the trisomic situation using the flected translocation.

ROBERT P. DIAMOND
ROBERT P. ERICKSON

Department of Pediatrics,
University of California,
San Francisco,
San Francisco, California 94143

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Amelioration of Marek's disease and absence of vaccine protection in immunologically deficient chickens

MAREK'S disease (MD) is a lymphoproliferative disease of chickens which is generally considered to be neoplastic¹. It is probably the most commonly occurring neoplasm of any animal and until recently it caused extensive economic loss, estimated to be in excess of 150 million dollars annually, to the poultry industry². Several viral vaccines which will effectively prevent the disease have been developed³ and the most extensively used is a herpesvirus isolated from turkeys (HVT)⁴, which will prevent the disease in laboratory and field trials^{5,6}.

The characteristics of the protection are unusual. On vaccination, chickens develop persistent viraemia with HVT.

Also, vaccination with this virus does not prevent infection of the host with virulent MD virus (MDV), replication and persistence of the virulent virus in the host, and shedding of the virulent virus from the host^{6,7,8} albeit at a slightly lower level. Thus, the protection cannot be mediated entirely by immune mechanisms directed against virus replication and shedding. A variety of mechanisms have been proposed^{9,10,12}.

Cells constituting MD lymphomas could have originated from bursa, thymus or bone marrow¹. From antigenic marker studies, they are apparently predominantly thymus-derived lymphocytes^{12,13}. Also, neo-natal bursectomy does not interfere with MD induction¹⁴, and thymectomy decreased the proportion of genetically susceptible birds that died of MD or had MD lymphomas¹. The thymus cells of the tumour may have two roles: as a source of proliferative target cells or as a mediator of cellular immune response against the target cells. We show here that cyclophosphamide (CP) treatment significantly reduces the incidence of MD. Also, immunologically deficient chickens bursectomised with high doses of CP were not protected against MD by vaccination with HVT.

Chickens, highly susceptible to MD, were line 7 in experiment 1 or a cross between line 15 males and line 7 females in experiment 2 (ref. 15). The parent flocks are known to have antibody to MDV that is transmitted passively to the progeny. Progeny hatched and reared in isolation, however, are free of MDV and do not acquire antibody to MDV. All chicks were reared in modified Horsfall Bauer isolators. For virus isolation, approximately 2×10^7 spleen cells were inoculated on confluent DEF cultures prepared and maintained as described¹⁶. Plaques were counted 14 d after inoculation. Antibody to MDV was assayed by the agar gel precipitation test with antigen prepared in duck embryo fibroblasts¹⁷ or by the indirect fluorescent antibody test¹⁸.

To test for the ability of the chickens to produce antibody to sheep erythrocytes (SE), we inoculated groups of chickens at 4, 6 or 7, and 9 weeks of age with 1×10^8 to 2×10^8 erythrocytes at each of five sites per chicken. Serum was collected at the end of the experiments (8 weeks for experiment 1 and 11 weeks for experiments 2 and 3), and antibody was assayed by haemagglutination¹⁹. All birds dying during the experiment and all those that survived were examined for MD lesions by gross and histopathological methods²⁰. For chemical bursectomy, chicks were inoculated intramuscularly on the first 4 d of life with either 4 or 5 mg of CP d⁻¹ (Cytosan, Mead Johnson Laboratories, Evansville, Indiana). In previous experiments (H.G.P., unpublished), chicks of line 15 $\times$ 7 inoculated with a total of 16 mg of CP were unable to produce antibody to SE, *Brucella abortus*, and bovine serum albumin, and most of the chicks were agammaglobulinanaemic. The HVT strain FC126 in its eleventh duck embryo fibroblast passage⁴ was administered on day 6. The JM strain⁵ and cloned preparation of the GA strain²¹ of MDV, which were highly pathogenic, were used to induce

TABLE 1 Effect of cyclophosphamide on Marek's disease (Experiment 1).

CP	Treatment MDV	No. of Chickens	% Marek's disease response			Virus isolation	Antibody to:	
			Dead*	Gross†	Total‡		MD	SE
16 mg	7 d	8	38	50	100	3/3	0/3	
16 mg	—	7	0	0	14§			0/7
—	7 d	12	67	83	92	1/4	4/4	
—	—	14	0	0	14§		0/5	13/13

CP, Cyclophosphamide administered intramuscularly, 4 mg d⁻¹ for the first 4 d after hatching; MDV, Marek's disease virus, JM strain, inoculated intra-abdominally, 1,100 plaque-forming units (PFU) per chick at 7 d of age; SE, sheep erythrocytes injected intramuscularly (10^9 cells per chick) at 4 and 6 weeks of age and tested at 8 weeks. Antibody to SE was measured by haemagglutination. Antibody to MDV was measured by the indirect fluorescent antibody test.

* % Chickens dying of MD.

† Dead plus survivors that had gross MD lesions at the end of the experiment at 8 weeks of age.

‡ Gross plus survivors that had microscopic MD lesions.

§ Chickens in these groups had minor histological lesions indistinguishable from those caused by MDV.

TABLE 2 Effect of cyclophosphamide on Marek's disease and on protection offered by the herpesvirus of turkeys (Experiment 2).

CP	Treatment		No. of Chickens	Dead*	% Marek's disease response		Total†
	HVT	MDV			Gross‡		
20 mg	6 d	3 weeks	10	50	50		90
20 mg	—	3 weeks	16	31	38		69
20 mg	—	6 d	9	33	67		89
20 mg	—	—	11	0	0		0
—	6 d	3 weeks	10	0	0		0
—	—	3 weeks	12	42	67		92
—	—	6 d	15	67	80		100
—	—	—	14	0	0		0

CP, Cyclophosphamide administered intramuscularly, 5 mg d⁻¹ for the first 4 d after hatching; HVT, the herpesvirus of turkeys inoculated intra-abdominally, 3,700 (PFU) per chick at 6 d of age; MDV, Marek's disease virus, GA strain, inoculated intra-abdominally, 33,000 (PFU) per chick at 6 d of age or 56,000 (PFU) per chick at 3 weeks of age. Experiment ended at 11 weeks of age.

* , † , ‡ See Table 1.

MD by inoculation on the sixth to seventh day of age or to challenge immunity by inoculation on day 21,

Treatment with 16 mg (Table 1) or 20 mg (Tables 2 and 3) of CP significantly reduced mortality ($P \leq 0.01$ when data from both times of exposure are pooled and compared in a one-tailed χ^2 test) and gross lesions ($P \leq 0.01$) of MD even when MD virus was given 3 weeks after CP treatment (Tables 2 and 3). When histological lesions were included, the differences were not significant. The CP-treated chickens tested were infected with MDV as the virus was isolated from most birds tested (Table 3) and most had MD lesions. None of those tested could respond with antibody to either viral antigens or SE. They were therefore deficient in bursa-dependent lymphoid functions.

Experiments 2 and 3 were designed to determine whether CP would affect protection by HVT. In both experiments, vaccinated birds not treated with CP, whose immunity was challenged with the GA (Table 2) or JM (Table 3) strains of MD at 3 weeks of age, were fully protected, those treated with CP had up to 90% MD, depending on the criteria used to measure the response. Thus, these chickens lacked the immunity to MD conferred on normal chickens by HVT. Virus re-isolated from both CP-treated and untreated chickens at the end of the experiment showed that they had become infected with HVT. Although some chickens died, most survived. These surviving chickens were unable to produce antibody to viral antigens. We conclude that they were deficient in their bursa-dependent lymphoid functions.

Among the two control groups not inoculated with MDV (Table 1) and one group inoculated with HVT (Table 3), 1, 2, and 2 birds, respectively, had mild lymphoid infiltrations in the nerves. This type of lesion has been described previously in isolated controls^{7,18,22} and is considered to be unrelated to MDV infection. The presence of viral plaques in cultures from two groups of birds that had not been inoculated with either MDV or HVT (Table 3) cannot be

explained because these groups of birds also lacked antibody to viral antigens.

Treatment with CP eliminated bursa-dependent immune functions and caused a temporary lymphoid depletion of the thymus²³. By 15 d, however, the appearance of the thymus had returned to normal, and at 1 month (the earliest time tested) it was functionally competent. Because surgical bursectomy and irradiation did not affect MD¹⁴ and because bursa-dependent lymphoid cells were only a small part of MD lymphomas^{12,13}, it was unlikely that the effect of CP on the bursa would have been responsible for the observed reduction in MD. Because thymectomy reduced MD lymphomas in susceptible chickens, the effect of CP on the thymus may possibly have accounted for the reduction in MD, particularly because a high dose of CP was used. Prolonged reduction of thymus-dependent function was not observed²³ however. Tests of thymus function during the development of MD in CP-treated chicks would be helpful in determining the role of the thymus. Also CP may possibly have affected bone marrow cells which may be involved in the pathogenesis of MD (ref. 1). A combination of these effects may also have occurred.

Protection against MD by HVT was largely absent in CP-treated chickens even though immunity was challenged when birds were 3 weeks old. The absence of protection was most likely due to the obliteration of bursa-dependent lymphoid cell function by CP although CP may also have had an effect on thymus function. The effect of CP on thymus function was not sufficient to prevent MD completely; some thymus target cell function or cellular immune response to target cells may have persisted. The role of the thymus-dependent immune system in immunity and the effect of CP on it are therefore still undetermined. If the bursa-dependent lymphoid system played a major role in protection induced by HVT vaccine, its effect was probably mediated through antibody to the tumour cell or virus-infected cell rather than through

TABLE 3 Effect of cyclophosphamide on Marek's disease and on protection offered by the herpesvirus of turkeys (Experiment 3).

CP	Treatment		No. of Chickens	% Marek's disease response			Virus isolation	Antibody to:	
	HVT	MDV		Dead*	Gross‡	Total†		MD	SE
20 mg	6 d	—	11	0	0	20§	5/7	0/7	
20 mg	6 d	3 weeks	11	0	45	55	6/7	0/7	
20 mg	—	3 weeks	6	17	50	83	5/6	0/6	
20 mg	—	—	7	0	0	0	1/7¶	0/7	0/7
—	6 d	3 weeks	10	0	0	0	6/7	7/7	
—	—	3 weeks	14	57	86	93	5/6	6/6	
—	—	—	10	0	0	0	1/7¶	0/10	10/10

CP, Cyclophosphamide administered intramuscularly, 5 mg d⁻¹ for the first 4 d after hatching; HVT, the herpesvirus of turkeys inoculated intra-abdominally, 4,000 PFU per chick at 6 d of age; MDV, Marek's disease virus, JM strain, inoculated intra-abdominally, 690 PFU per chick at 3 weeks of age; SE, sheep erythrocytes injected intramuscularly, 5×10^8 cells per chick at 4 and 6 weeks of age. Antibody to SE was measured by haemagglutination. Antibody to MDV was measured by the agar gel precipitation test. The experiment ended at 11 weeks of age.

* , † , ‡ See Table 1.

§ Both chickens had minor histological lesions indistinguishable from those caused by MDV.

¶ Plaques in one of two replicates possibly due to procedural error.

antibody neutralising the virus. This is because cell-free virus accessible to neutralisation by antibody cannot be demonstrated in blood and internal tissues of MDV-infected chickens. The absence of an effect of vaccine-induced immunity on infection, replication, and shedding of the virulent virus from the host may also have resulted from the highly cell-associated nature of MDV within the body of the chicken. Nevertheless, we conclude that an immunosuppressive drug will eliminate HVT vaccine protection against MD.

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H. G. PURCHASE
J. M. SHARMA

United States Department of Agriculture
Regional Poultry Research Laboratory
3606 East Mount Hope Road
East Lansing, Michigan 48823

Received November 19, 1973.

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diploid cell lines. What seems to take place, however, is an increased transit time and heterogeneity of cell cycles^{4,5}.

The first experiments that analysed the kinetics of cell division of human fibroblasts at different passage levels^{4,5} showed a decrease in the number of cells which synthesised DNA during a 24 h labelling period and after a continuous labelling between subcultivation and confluency. This seemed to be caused by a prolongation of the generation time due to a delay in the G₁ and G₂ periods of the division cycle. It should be stressed that these experiments referred only to the time between a subcultivation and confluency and did not consider the possibility that cells which did not divide during one passage might do so in the following one. It was subsequently reported⁶ that the percentage of cells capable of division decreased with increasing age in tissue culture. The latter experiments, however, were done by plating 200 cells per 60 mm Petri dish so that each cell remained isolated, so it reveals the number of cells capable of dividing when cloned. This is completely different from how a cell behaves when in the proximity of other cells. It has also been shown⁷ that the percentage of cells capable of entering DNA synthesis after a 30 h labelling period at each passage of human diploid cell lines declines as a function of either time or population doublings. This technique also allows the evaluation of the age of the culture, which is much more precise than referring to the passage number.

These reports⁴⁻⁷ form the basis for the concept that non-cycling cells are present in increased amounts during cell senescence *in vitro*. The cells that did not synthesise DNA were not followed^{4,5,7} in subsequent passages however, and Merz and Ross⁶ analysed cell division in an environment unfavourable to the cell.

When phase II (20th passage) HEF were subcultivated with different split ratios, (cells from one flask were subcultivated by transfer into either another flask (1:1), into two flasks (1:2), or into 4 flasks (1:4)), and were labelled continuously, 21% of the cells synthesised DNA in the first group, 75% in the second, and 97% in the third group (Table 1). This shows that with higher inocula (1:1 and 1:2 splits) not all the cells have time to enter DNA synthesis before growth stops. After a 1:4 split however, almost all cells had time to enter the division cycle before they were affected by the mechanisms responsible for the inhibition of division during cell crowding. Thus, the results of an experiment with only a 1:2 split (which is the way these cells are usually carried) would have suggested that at this passage there are 25% non-dividers.

When HEF very near the end of their lifespan *in vitro* are labelled continuously with tritiated thymidine (³H-TdR) from the time of seeding to the time when further growth ceases, and are analysed by autoradiography, 66% of the nuclei become labelled during that time (Table 2). If identical cultures are subcultivated after that period and are again labelled continuously with ³H-TdR, 7 d after the second subcultivation 86% of the nuclei are labelled and 2 weeks later 92% of the cells are labelled. (The cultures were confluent from 17 d.) Thus only 8% of the cells remained unlabelled after the two last passages of this HEF line. This cannot be due to dilution of the non-dividers by dividing cells, for even if all the cells that had entered DNA synthesis

Are non-dividing cells present in ageing cell cultures?

THE idea that an increased number of non-dividing cells accumulate during the growth decline of human embryonic fibroblasts (HEF) cultivated *in vitro* has been put forward¹. This concept of a non-dividing fraction of cells seems to be widely accepted^{2,3}, but no compelling evidence exists so far, to show that non-cycling cells exist in old cultures of human fibroblasts. On the contrary, it seems that most, if not all, the cells can divide up to the end of the lifespan of human

TABLE 1 Percentage of cells which synthesised DNA between subcultivation and resting phase after different split ratios

Split ratios	Percentage
1:1	21
1:2	75
1:4	97

Cultures were labelled continuously during 4 days with 0.01 μ Ci ml⁻¹ ³H-TdR (specific activity (1.9 Ci mM⁻¹))

TABLE 2 Percentage of cells which synthesised DNA during the 45th and 46th subcultures of an HEF cell line

Days after subcultivation when duplicate cultures were fixed	Cells	
	Between the 45th subculture and confluency (%)	Between the 46th subculture and confluency (%)
11	66	
7		86
11		86
14		92
17		94
24		92
29		92

Cultures were labelled continuously by adding 0.01 $\mu\text{Ci ml}^{-1}$ specific activity 1.9 Ci mM^{-1} every 4d.

had divided, the non-dividing fraction would be diluted to 20%. Also, it was previously shown^{4,5} that in late passages the population does not double because many of the cells that enter DNA synthesis take a long time to reach mitosis, because they are delayed in the G_2 period.

With chick fibroblasts, nearly 100% of the cells divide between each subcultivation and confluency, in human fibroblasts however, a large proportion of the cells do not enter DNA synthesis during one passage, but do so during the following passage. This difference between human and chick cells could be explained on the basis of the increased heterogeneity of the cell cycles in human cells. As a result of this heterogeneity of generation times, when human cells reach saturation density some cells divide one or two times (short cycles) and others do not have time to complete their cycle⁶. In old populations, since this heterogeneity is more pronounced⁴, more cells are unable to complete the cycle before saturation density is reached. In chick fibroblasts, however, although the generation time also seems to be prolonged⁸, it is perhaps uniformly prolonged throughout the whole population, so that at each passage every cell has an equal chance of completing the division cycle before growth stops.

Whatever the reasons, in the systems studied so far, non-cycling cells do not seem to be a feature of cell ageing *in vitro*.

A. MACIEIRA-COELHO

*Institut de Cancérologie et d'Immunogénétique, (INSERM)
Hôpital Paul-Brousse,
94800 Villejuif*

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this is the first description of a cloned cell line that exhibits a growth response to a physiological dose of androgen. The mechanism of action of the steroid may be similar to that in the normal tissue of origin.

A transplantable leiomyosarcoma (Accession No. 14072, Experimental No. LX-7330-MU9-TB-MU1) from Syrian hamster ductus deferens tissue was obtained^{1,2} in March 1971. The original tumour from which the DDT₁ cells were established was induced in male hamsters by long-term administration of diethylstilboestrol (DES) and testosterone². The tumour was autonomous when received but showed some T-binding activity (J. S. N., unpublished observation). The sarcoma was dispersed by trypsinisation and placed in culture in Ham's F-10 with 10% horse serum, 5% foetal calf

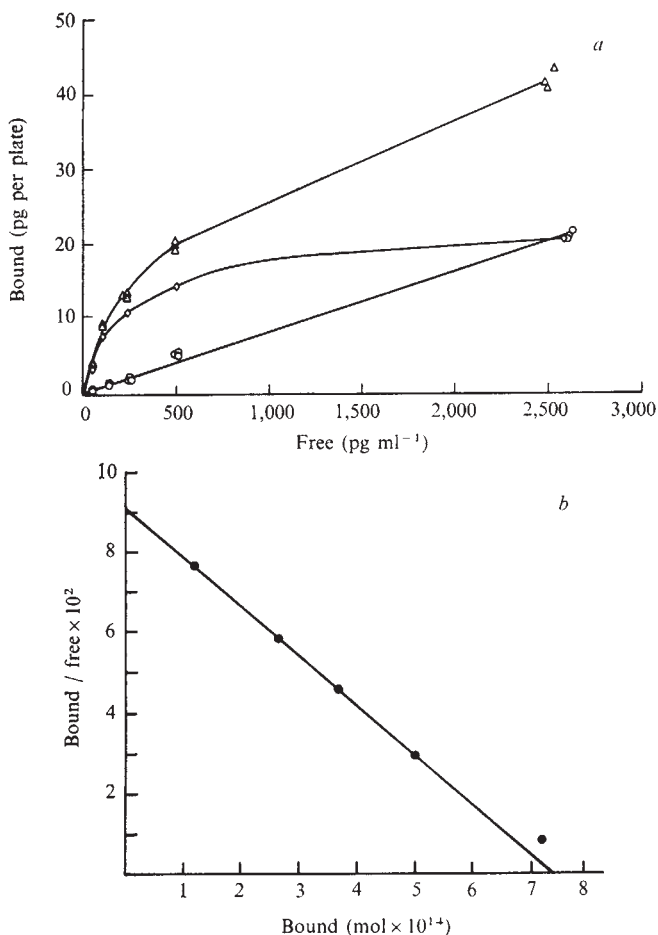


FIG. 1 *a*, Cells grown in roller culture were collected by shaking, pelleted at 800*g*, and washed 2 h in minimal essential medium (MEM) buffered with HEPES pH 7.6 at 4° C. Aliquots of cells in 10 ml of MEM were distributed evenly to 25 ml Erlenmeyer flasks and warmed to 37° C. 1, 2, 6, 7 ^3H -testosterone (Specific activity, 91 Ci mmol^{-1} New England Nuclear) dissolved in ethanol-MEM 1:1 was added at varying concentrations either alone or in combination with fifty-fold excess testosterone. At no time did the ethanol exceed 0.1%. Incubation was terminated at 75 min by withdrawing samples and immediately placing them on ice (all further operations are at 4° C unless indicated). All samples were centrifuged at 1,400*g* and washed twice with Tris saline pH 7.6. Bound ^3H -testosterone was extracted into 3 ml of absolute ethanol (at 21° C) and counted in 10 ml of a toluene based scintillation fluid (4 mg PPO + 50 mg POPOP per l toluene). Specific binding was calculated by subtracting non-specifically bound testosterone (^3H -testosterone + fifty-fold excess testosterone) from total bound testosterone (^3H -testosterone). Free ^3H -testosterone was measured directly from the incubation medium after centrifugation to remove the cells. Protein was determined by the method of Lowry³. Δ , Total; $\diamond$, specific; $\circ$, non-specific. *b*, Binding analysis was performed by the method of Scatchard⁹ and only specifically bound was plotted.

Androgen receptors in a Syrian hamster ductus deferens tumour cell line

We have established in culture a cloned line of hamster ductus deferens cells that contains a saturable, high affinity testosterone receptor protein. These cells exhibit an increased rate of DNA synthesis in response to testosterone and 5 α -dihydrotestosterone (DHT) treatment, although they probably do not require either androgen for survival. We believe

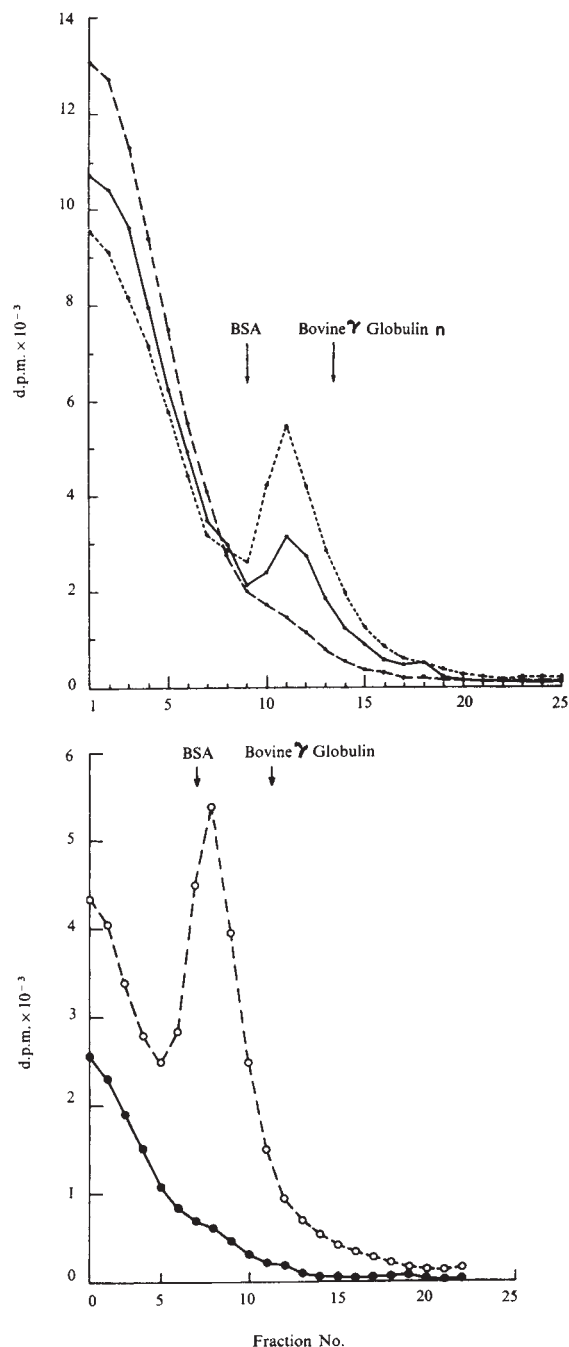


Fig. 2 *a*, Sucrose gradient analysis was performed in linear 5–20% sucrose made up in 10% glycerol containing 20 mM Tris, 100 mM KCl, and 10^{-6} M phenyl methyl sulphonyl fluoride (PMSF) at pH 7.45. Gradients (4.8 ml) were prepared with a Buchler University Gradient Former and 0.2 ml fractions of 150,000g cytosol containing 2.3 mg protein were layered just before centrifugation at 45,000 r.p.m. for 17 h at 2° C in the Beckman SW 50.1 head. Five drop fractions were collected and counted at 25% efficiency in 13 ml of scintillation fluid (1 l of toluene + 4 gm PPO + 50 mg POPOP diluted to 1.3 l with absolute ethanol). Cytosol was prepared by homogenising the cells with 15 strokes of a dounce type B in 20 mM Tris, 2 mM MgCl_2 , 2 mM CaCl_2 , 0.5 mM mercaptoethanol, 1 μM PMSF, and 10% glycerol pH 7.45 (buffer A) at 2° C. The homogenate was centrifuged at 150,000g (Ave) in a Beckman type 65 head for 45 min at 2° C. 1, 2, 6, 7 ^3H -testosterone (Specific activity, 91 Ci mmol $^{-1}$, New England Nuclear) was added to buffer A to a concentration of 1.9×10^{-8} M and enough cytosol was added to dilute this to 1.9×10^{-9} M. Unlabelled steroids (testosterone or E_2) were added at a fifty-fold concentration (1×10^{-7} M) before addition of the cytosol. All the samples were incubated at least 2 h before centrifugation, (.....), ^3H -testosterone; (-----), ^3H -testosterone + testosterone; (—), ^3H -testosterone + E_2 . Sedimentation values were calculated by the method of Martin and Ames⁷ using bovine serum albumin (4.6S) and γ -globulin (7.0S) as standards. *b*, Whole cells were incubated for 1 h at 37° C in Dulbecco's Modified Eagle's medium (DME) with 25 mM HEPES buffer containing 6×10^{-9} M 1, 2, 6, 7 ^3H -testosterone ($\circ\text{---}\circ$) or 6×10^{-9} M 1, 2, 6, 7 ^3H -testosterone + 6×10^{-7} M testosterone ($\circ\text{---}\circ$). The cells were then washed twice in 3 ml Tris-saline pH 7.45 (all further operations at 2° C) in 12 ml conical centrifuge tubes and always centrifuged 5 min at 700g. The cells were washed a third time in buffer B (buffer A with 0.25 M sucrose instead of glycerol) and pelleted at 1,400g for 5 min. The cell pellet was transferred to a dounce type B and an equal volume of buffer B was added before homogenisation by 15 strokes. The resulting homogenate was centrifuged at 1,400g for 15 min. The nuclear pellet was resuspended by gentle vortexing and washed twice with 2 ml of buffer B. The nuclei were then diluted with an equal volume of buffer C (buffer A with 800 mM KCl) and extracted over 2 h with occasional gentle vortexing. The nuclear extract was centrifuged at 2,500g for 10 min and 0.2 ml of the supernatant containing 2.0 mg soluble protein was analysed on sucrose gradients (with the exceptions that the gradients contained 400 mM KCl) and then counted as described in (*a*).

serum with 100 units ml^{-1} penicillin, 100 μg ml^{-1} streptomycin, 2.5 μg ml^{-1} fungizone (Gibco) and 1×10^{-9} M testosterone (Sigma). On reimplantation of the cells from culture into non-syngeneic Syrian hamsters, a new tumour developed and grew at essentially the same rate as the original. Since the tumour might consist of a heterogeneous cell population with a small number of cells responding to testosterone, a second trypsinisation was performed and the resulting cells were cloned in the presence of 1×10^{-9} M testosterone using Falcon microtest plates³. Cells from each clone were frozen in growth medium with 10% DMSO and saved for later analysis. Of the clones analysed to date, one designated DDT₁, has been shown to have a high steroid binding activity. The DDT₁ cell line shows several characteristics of a permanent cell line such as log-phase growth in suspension culture and formation of tumours on reinjection into animals.

Several experiments have now shown that the DDT₁ cells respond to a physiological dose of testosterone or DHT by

increasing the rate of synthesis of DNA, as measured by incorporation of ^3H -methylthymidine (^3H -Tdr) into the hot perchloric acid soluble fraction of the cell (Table 1).

Since testosterone seems to stimulate the growth of the DDT₁ cells, we examined ^3H -testosterone retention in an intact cell system (Fig. 1). These studies indicate the presence of a high affinity receptor ($K_d = 8.5 \times 10^{-10}$ M) which is saturable at 3.6×10^{-14} M per mg protein. There are approximately 3,400 binding sites per cell.

Having demonstrated a high affinity, saturable retention of ^3H -testosterone with whole cells, we analysed this finding in a cell-free extract. Sucrose gradient analysis of 150,000g cytoplasm from the DDT₁ cells has repeatedly demonstrated a receptor binding ^3H -testosterone or its metabolites that sediments at 5.6–5.8s in 5–20% sucrose gradients made up in 10% glycerol as described in Fig. 2a. This binding is completely abolished when unlabelled testosterone is included in the incubation mixture. E_2 reduces the binding by 33.5%. Enzymatic degradation of the material in this fraction by trypsin (500 μg ml^{-1}) at 2° C for 2 h, but not RNase (500 μg ml^{-1}) or DNase (500 μg ml^{-1}), suggests that the receptor is a protein.

When whole cells are incubated 1 h in the presence of ^3H -testosterone, a receptor can be extracted from the nuclear pellet using a high salt buffer (Fig. 2b). The receptor sediments at approximately 5.1S and when unlabelled testosterone is added to the incubation media the 5.1S peak disappears.

The DDT₁ cell line seems to be the first cloned cell line that exhibits a growth response to testosterone and DHT

TABLE 1 Effect of DHT and testosterone on the rate of DNA synthesis in cell culture

	Mean μg DNA per plate* at day 4	P	Mean d.p.m. ^3H -Tdr per μg DNA*	P
Control	31.92 $\pm$ 2.78		384.4 $\pm$ 16.4	
8.9×10^{-9} M 5α DHT	40.88 $\pm$ 0.99	<0.001	525.7 $\pm$ 27.6	<0.001
8.9×10^{-9} M Testosterone	37.72 $\pm$ 1.67	<0.005	466.5 $\pm$ 9.6	<0.001

* Values $\pm$ s.d.

At the time the androgens were added there were 9.7 ± 0.4 μg DNA per plate.

Replicate cultures were plated at a density of 5.75×10^5 cells per 60 mm tissue culture dish in Ham's F-10 (all media and chemicals from Gibco unless otherwise stated) with 1% penicillin-streptomycin solution, 1% fungizone, and 10% foetal bovine serum. The serum had been heated for 1 h at 55°C in the presence of 3% by weight activated charcoal (G-60, Matheson Scientific) and filter sterilised. Steroids were not added for 24 h to avoid any effect the androgens might have on plating efficiency. On day 2 all cultures were changed and androgens were added as designated. The control group received 0.01% carrier ethanol but no added steroids. The androgen-treated cultures received the same medium but with the addition of 8.9×10^{-9} M testosterone or DHT. Thereafter, the culture media were changed every 24 h for 4 d. Any cells floating in the expended media were retrieved by centrifugation and saved for inclusion in the final DNA totals. ^3H -methyl thymidine (^3H -Tdr) (NET-027Z Specific activity 43 Ci mmol $^{-1}$) was added on day 4 directly to the cultures in 0.05 ml of Hanks' balanced salt solution to a final concentration of $0.4 \mu\text{Ci ml}^{-1}$. After incubation for 2 h the cells were collected by scraping, and washed twice with ice-cold Tris-saline pH 7.4. All centrifugations were at 800g. The cold acid-soluble counts were removed with three 3 ml 10% TCA washes at 0°C . The cells were then incubated at 70°C for 30 min in 0.5 N PCA. After centrifugation a 0.2 ml aliquot was counted for tritium as described in Fig. 2a. DNA analysis was performed by the method of Burton⁶. Probability was calculated using Student's *t* test of means. In all groups five samples were used.

which also contains cytoplasmic and nuclear androgen receptors^{10,11} although Smith *et al.*¹² have described androgen-responsive mammary tumour cells which bind DHT. The cytoplasmic protein in some respects resembles androgen binding protein from other cells but is different from the binding protein found in sera^{4,8}. Furthermore, the steroid binding protein in the DDT₁ cells seems to be involved in the nuclear translocation of testosterone or its metabolites to a nuclear acceptor. Therefore, we conclude that the DDT₁ cell line will prove a useful tool in the search for the mechanism of action of androgens.

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JAMES S. NORRIS

Department of Cell Biology,
Baylor College of Medicine,
Houston, Texas 77025

JACK GORSKI

Departments of Biochemistry
and Animal Sciences,
University of Wisconsin 53706

PETER O. KOHLER

Departments of Medicine
and Cell Biology,
Baylor College of Medicine,
Houston, Texas 77025

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Serotonin and pituitary-adrenal function

SEVERAL reports have indicated a relationship between the pituitary-adrenal secretion of steroids and brain serotonin (5-hydroxytryptamine) in the rat^{1,2}. Serotonin is believed to be a transmitter or regulator of neuronal function. We have previously investigated the effects of altering brain serotonin concentrations on the daily fluctuation of the pituitary-adrenal system³. We have investigated this problem further, by evaluating the response of the pituitary-adrenal system to a stress stimulus in the rat. Our approach was to either inhibit brain serotonin synthesis with *p*-chlorophenylalanine (PCPA), or to raise the concentration of serotonin with precursors such as tryptophan or 5-hydroxytryptophan (5-HTP)⁴.

We used male Sprague-Dawley rats (150–200 g). They were kept in a 12L:12D environment (lights on at 0700 h) and given food and water *ad libitum*. Serum corticosterone was assayed fluorometrically, while the determination of serum ACTH was made by bioassay⁵. The concentrations of brain serotonin were determined after isolation, using a weak cation exchange resin and fluorometric assay⁶. One hundred and eighty rats divided into two groups were injected intraperitoneally (i.p.) with pyrogen-free saline or PCPA (Pfizer) in saline at a dose of 300 mg kg $^{-1}$ d $^{-1}$.

Daily injections of PCPA for 4 d greatly enhanced and sustained the peak ACTH response to ether stress ($P < 0.05$) (Fig. 1). To assess whether this enhanced response was due to an effect of PCPA on the diurnal variation in

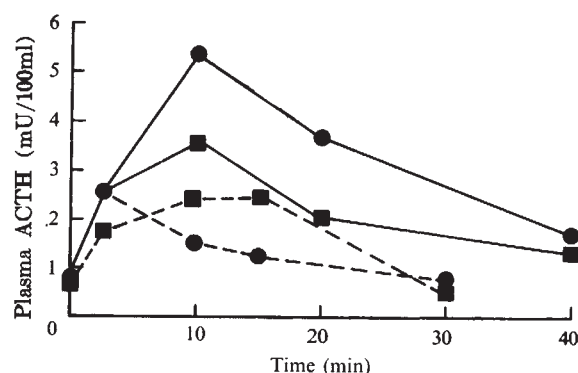


FIG. 1 Time course of the ACTH response to 1 min of ether stress. Each value represents the mean of eight animals. ●, Morning; ■, evening; —, PCPA; ---, saline.

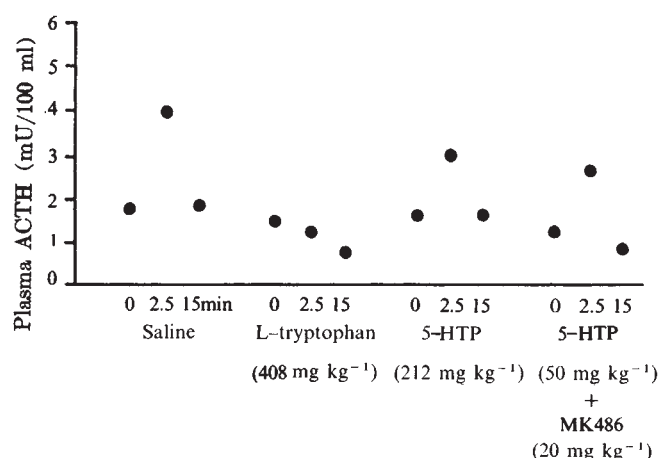


Fig. 2 Effect of serotonin precursors on the ACTH stress response. L-Tryptophan (408 mg kg^{-1}) or 5-HTP (212 mg kg^{-1}) or 5-HTP (50 mg kg^{-1}) with the peripheral decarboxylase inhibitor, L- α -hydrazinomethyl-dihydroxyphenylalanine (MK 486; 200 mg kg^{-1}) were injected i.p. into groups of rats (eight per group). The animals were adrenalectomised 24 h before the study to enable us to study the ACTH response isolated from the feedback effects of circulating steroids. The rats were studied 2 h after injection of the serotonin precursors when brain serotonin levels have been reported at maximal levels after the injection of precursors¹¹ and when pituitary adrenal activity has usually returned to normal in control animals after the stress of an injection. Animals were either decapitated and bled at zero time without ether stress or decapitated and bled 2.5 or 15 min after the stress of ether.

the rate of secretion of ACTH in response to stress, the time course of this response to 1 min of ether stress was determined at 0800 h or 2000 h. Figure 1 shows that, whereas in the controls, blood ACTH concentrations after stress peaked at 2.5 min in the morning and at about 15 min in the evening, depletion of serotonin not only enhanced the response, but also greatly altered the rhythm in the response to ether stress; maximum stress response occurred at 10 min, both in the morning and in the evening. Thus, inhibition of brain serotonin synthesis enhanced the stress response, in addition to altering the pattern of the pituitary-adrenal stress response. This agrees with our previous findings that daily injections of PCPA for 2–4 d raised the morning low, and prevented the evening rise in plasma corticosterone in unstressed animals^{1,3}.

Our alternative approach to the study of the role of brain serotonin in the regulation of pituitary-adrenal function was to attempt to raise brain serotonin by the use of the precursors tryptophan (J. T. Baker Co) and 5-HTP (Calbiochem). Tryptophan is the dietary amino acid which is converted to 5-HTP, the immediate precursor of serotonin. We also used a decarboxylase inhibitor (MK-486, Merck, Sharpe and Dohme), which only acts peripherally in combination with 5-HTP to increase brain serotonin levels by inhibiting serotonin formation outside the brain. Adrenalectomised animals (eight per group) were stressed with ether 2 h after the administration of serotonin precursors, and the pattern of the rise in ACTH was studied (Fig. 2). In these adrenalectomised animals, tryptophan increased brain serotonin content by an average of 33%, and significantly reduced the stress-induced secretion of ACTH ($P < 0.01$) which, in the saline controls, occurred by 2.5 min, and returned to prestress levels by 15 min. 5-Hydroxytryptophan alone, or in combination with MK-486, increased brain serotonin content by 2.4-fold and 1.2-fold respectively, and reduced the stress response in these animals significantly ($P < 0.05$). Thus, these precursors of serotonin tended to decrease or inhibit the pituitary-adrenal response to stress. It is interesting that tryptophan which produced an

increase of about 33% in brain serotonin had a greater effect on ACTH than 5-HTP, which produced a much greater increase. This is consistent with the conclusion of Moir and Eccleston that tryptophan loading is the more physiological method of increasing brain serotonin content⁷.

Preliminary reports on human studies also appear to support this serotonin pituitary-adrenal interrelationship. 5-Hydroxytryptophan seems to reduce the 24 h excretion of 17-hydroxycorticosteroids in human volunteers^{3,8}.

It is now reasonably well accepted that the pituitary-adrenal system functions as a closed loop. In this system, the positive vector or 'driving force' corresponds to environmental stimuli, acting through hypothalamic neuro-humoral pathways⁹ and the negative vector corresponds to the concentration of corticosteroids which act by feedback inhibition on the pituitary-adrenal system¹⁰. We found that the stress response was enhanced by inhibition of serotonin synthesis, and found indications of a reduction of the stress response by serotonin precursors. This suggests that serotonin may mediate or modulate this negative feedback mechanism regulating pituitary-adrenal function.

If this hypothesis were correct, then the inhibitory action of corticosteroids on steroid output would be expected to be less effective in animals in which brain serotonin was depleted. Figure 3 illustrates the results of an experiment testing this possibility, and compares the corticosterone stress response inhibiting properties of two doses of a steroid (prednisolone) in control rats and in animals whose brain serotonin content was markedly reduced by pretreatment with PCPA. Both doses of the steroid effectively inhibited the response to the stress of ether and laparotomy in the control animals. In serotonin-depleted animals, neither dose was capable of blocking the stress response, further indicating that serotonin is indeed involved in the negative feedback to the pituitary-adrenal system.

These results, taken together, lead us to speculate that in some disease states, such as Cushing's syndrome, where there is a similar inability of corticosteroid to suppress the pituitary-adrenal system, there is a defect in serotonergic neuronal processes that impairs pituitary-adrenal feedback mechanisms. Thus, we reason that serotonin precursors may be useful as a diagnostic screening test to identify cases of Cushing's syndrome with the postulated serotonergic defect. Further, serotonin precursors (tryptophan or 5-HTP, with

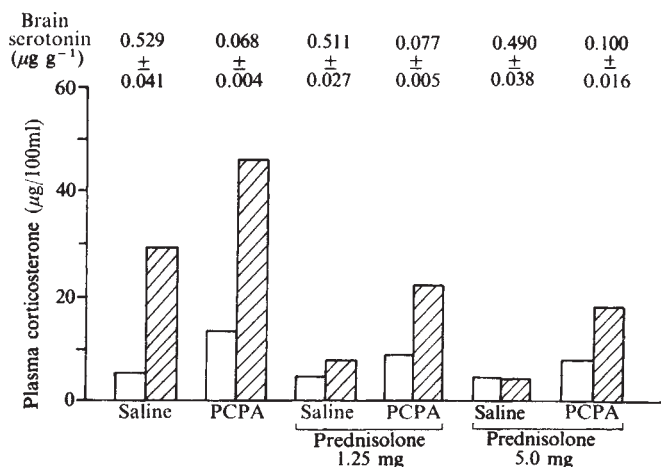


Fig. 3 The effect of serotonin depletion on the inhibition of corticosterone release by prednisolone. Groups of rats (eight per group) were stressed with ether and laparotomy 24 h after two daily injections of PCPA ($300 \text{ mg kg}^{-1} \text{ d}^{-1}$ i.p.) or saline and 4 h after the injection of the steroid (1.25 mg or 5.0 mg per 100 g body weight subcutaneously). Brain serotonin concentrations were determined on the unstressed group of rats. Open columns, unstressed; hatched columns, 15 min after ether laparotomy stress.

or without a peripheral decarboxylase inhibitor), or agents which stimulate serotonin receptors, may be useful in the therapy of some forms of Cushing's disease.

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PHILIP A. BERGER

JACK D. BARCHAS

Department of Psychiatry,
Stanford University School of Medicine,
Stanford, California 94305

JOAN VERNIKOS-DANELLIS

Human Studies Branch,
NASA/Ames Research Center,
Moffett Field, California 94035

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Unresponsiveness of human B lymphocytes to phytohaemagglutinin

A WIDELY accepted, but unproven, distinction between human T (thymus-derived) and B (non-thymus-derived) lymphocytes is their differential response to mitogenic stimulation with phytohaemagglutinin (PHA). It has been claimed that such a distinction does not truly exist and that the human B cell, unlike the murine B cell, transforms in the presence of PHA¹. An editorial² commenting on the consequences of this claim appropriately emphasised the danger of extrapolating data from one species to another. This communication presents evidence that pure populations of human B cells do not transform in response to stimulation with PHA. It also stresses the need of reducing the contamination of B cell preparations by T cells when studying the *in vitro* behaviour of the human B lymphocytes.

Pure populations of human T and B lymphocytes were obtained from tonsils using a two-step fractionation procedure. Cells were first fractionated on discontinuous gradients of bovine serum albumin (BSA)³ and then separated on the basis of their reactivity with the sheep red cell intermediates E and EAC1423 (EAC3) (Fig. 1).

Tonsil cells sedimenting in fraction 4 of the BSA gradient (interface of 23 and 25% BSA) were enriched in T cells. Forty to fifty per cent of the cells in fraction 4 formed rosettes with E (Fig. 1b), and only 15–20% reacted with EAC3 and stained with fluorescent antisera to human immunoglobulins (Fig. 1c, d). Cells sedimenting in fraction 7 of the BSA gradient (interface of 29 and 31% BSA) were almost exclusively B cells. Ninety to ninety-five per cent of

the cells in this fraction reacted with both EAC3 and fluorescent antisera to human immunoglobulins (Fig. 1c, d), and fewer than 5% formed rosettes with E (Fig. 1b).

B cells were obtained by taking the E non-rosette-forming cells from fraction 7. Ninety-eight to one hundred per cent of these cells exhibited reactivity with EAC3 and surface staining with fluorescent antisera to human immunoglobulins. Less than 2% of these cells formed rosettes with E on retesting (Table 1). T cells were obtained by taking either the

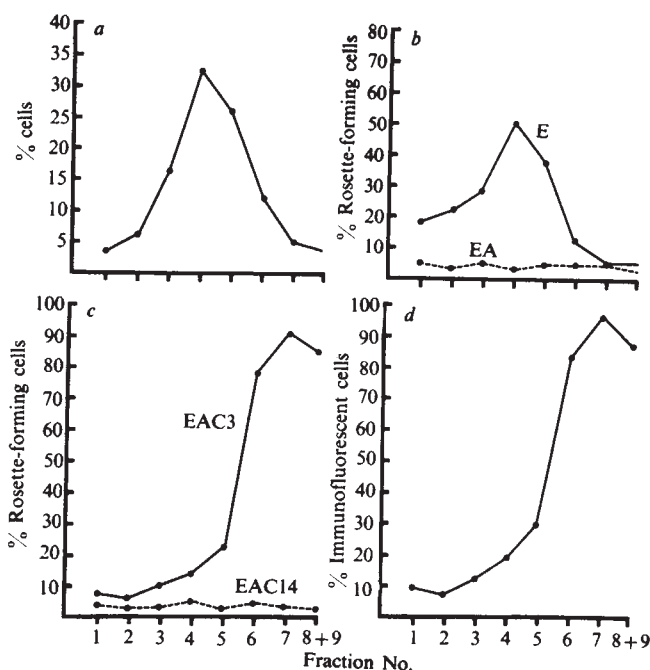


FIG. 1 *In vitro* response of tonsil lymphocytes separated into nine fractions by centrifugation in a discontinuous gradient of 17–35% BSA. *a*, Relative distribution of lymphocytes after purification by passage through glass beads. *b*, Rosette formation with sheep erythrocytes (E) compared to EA as a control. *c*, Rosette formation with EAC3 compared to EAC14 (control). *d*, Percentage of cells staining with antisera to human immunoglobulins. Values represent the average of four experiments. Human tonsils were teased in Medium 199 (Microbiological Associates), and the resulting suspensions were passed through columns of glass beads at 37° C to remove adherent cells. Column eluates contained more than 90% viable lymphocytes as determined by trypan blue exclusion. Cells in the eluate were fractionated on discontinuous gradients of BSA by a modification of the method of Dicke *et al.*³. Cells from each fraction were washed in Medium 199, then tested for their ability to form rosettes with the sheep red cell intermediates E and EAC1423, as well as for surface staining with fluorescent antisera to human immunoglobulins. Red cell intermediates E, EA, and EAC14 were prepared using human components of complement and were suspended at a concentration of 1% in phosphate-buffered saline (PBS). Lymphocyte suspensions were adjusted at 2×10^6 cells ml⁻¹ in PBS containing 10% serum from a donor with type AB Rh⁺ erythrocytes and absorbed three times with sheep erythrocytes. Equal volumes of lymphocyte and E or E-intermediate suspensions were mixed. Tubes containing E and EA were incubated for 30 min at room temperature, while those containing EAC3 and EAC14 were incubated for the same period of time at 37° C. Following this initial incubation, all tubes were centrifuged at 200g for 4 min at 4° C, and the pellets were incubated at 4° C for 1 h. Cells were then resuspended and examined microscopically, and the percent of rosette-forming cells determined. Lymphocytes intimately associated with four or more red cells were considered to have formed rosettes. Immunofluorescence was performed using fluorescein-conjugated antisera raised in goats against human IgG, IgM, and IgA myeloma proteins. Each antiserum was rendered monospecific for the heavy chain of a single class of immunoglobulins by absorption on suitable antigens coupled to Sepharose 4B by cyanogen bromide.

E rosette-forming cells or the EAC3 non-rosette forming cells from fraction 4. T cell preparations contained less than 5% EAC3-reactive immunofluorescent staining cells (Table 1).

B cells uniformly failed to transform in response to stimulation with PHA; stimulation index (s.i.) = 2 (Table 2). PHA caused vigorous proliferation of T cells with s.i. ranging from 57 to 166 (Table 2). The unresponsiveness of the B cell to PHA could not have been due to cell death. Viability of B cells after 72 h in culture always exceeded 65% as determined by trypan blue exclusion, and parallel B cell cultures always transformed normally in response to pokeweed mitogen (PWM) added at a concentration of 1/100 (s.i. 4-11).

Lack of adherent cells was ruled out as a cause for the unresponsiveness of the B cell cultures to PHA. Adherent cells, T cells, and B cells were prepared from the same tonsil. In combination experiments, 1×10^5 to 2×10^5 adherent cells were added to 1×10^6 B or T cells. The results in Table 2 show that the addition of adherent cells did not affect the response of the B cell to PHA, but increased the mitogenic response of the T cell to PHA. Adherent cells by themselves did not transform in response to PHA.

The effect of the contamination of purified B cell cultures by T cells on the PHA response was studied. Five per cent (5×10^5) T cells were mixed with 95% (95×10^5) B cells from the same donor. The overall response of the mixture to PHA was found to exceed by far the sum of the responses of the individual T and B cell components (Table 2). This effect was not seen when either one of the two components was previously subjected to irradiation with 7,000 r.

An attempt was made to determine if this enhancing effect was due to the action of a soluble mediator released by T cells and acting on B cells. The results in Table 3 show that supernatants from resting T cells, as well as supernatants from PHA-activated T cells, failed to cause transformation in B cells. T cells proliferated more vigorously in the presence of the PHA-containing supernatants than in the presence of PHA alone (Table 3).

That human B cells fail to transform with PHA was also shown by studying lymphocytes from a 6-month-old boy with the diagnosis of severe combined immunodeficiency. These lymphocytes exhibited characteristics of B cells; 3% formed rosettes with E; 95% showed immunofluorescence with an anti- μ -chain antiserum. Stimulation indices of 1.9 and 2.0 with PHA and 7.5 and 9.1 with PWM were obtained when these cells were cultivated *in vitro*.

The data presented here regarding the human B cell response to PHA are in agreement with those on B cells from

TABLE 2 Proliferative response of human B, T and adherent cells to PHA

Cells in culture	³ H-Thymidine incorporated per culture		s.i.
	Without PHA c.p.m.	With PHA c.p.m.	
1×10^6 B cells	1,011	1,332	1.3
1×10^6 T cells	864	96,722	112.0
1×10^6 adh cells	1,235	967	0.8
1×10^6 B cells + 1×10^6 adh cells	1,174	1,305	1.1
1×10^6 T cells + 1×10^6 adh cells	908	117,460	129.0
5×10^5 T cells	385	17,033	44.2
95×10^5 B cells	1,158	1,388	1.2
5×10^5 T cells + 95×10^5 B cells	1,147	38,565	33.6

Lymphocytes were cultured at a final concentration of 1×10^6 cells ml^{-1} in Medium RPMI-1640 (Grand Island Biological Company) supplemented with 15% serum obtained from an individual with AB Rh⁺ erythrocytes and containing penicillin ($50 \mu\text{g ml}^{-1}$), streptomycin ($50 \mu\text{g ml}^{-1}$), kanamycin ($50 \mu\text{g ml}^{-1}$), and mycostatin ($50 \mu\text{g ml}^{-1}$). Duplicate 1 ml cultures were set up in 12×75 mm Falcon plastic tubes and incubated in a humidified atmosphere of 5% CO_2 in air. Phytohaemagglutinin (PHA-P, Difco) was added at a final concentration of 1:100. Seventy-two hours after the onset of incubation, cultures were pulsed with ³H-thymidine (1.9 Ci mmol^{-1} , $1 \mu\text{Ci ml}^{-1}$; New England Nuclear). Sixteen hours later, cultures were collected and the amount of ³H-thymidine incorporated into DNA was determined by the filter paper disk technique². Results were expressed as c.p.m. incorporated per culture and as stimulation indices (s.i.) where

$$\text{s.i.} = \frac{(\text{c.p.m. of } ^3\text{H-thymidine incorporated by the stimulated culture})}{(\text{c.p.m. of } ^3\text{H-thymidine incorporated by the unstimulated culture})}$$

Adherent (adh) cells from tonsils were prepared by fractionating aliquots of single cell suspensions on BSA gradients without previous passage over glass beads. Fraction 3 of such gradients was enriched in monocytes (30-40%), determined by their ability to stain with neutral red. Cells from fraction 3 were reacted with E, after which the E non-rosette-forming cells were separated over a Ficoll gradient, washed, and resuspended at a concentration of $3 \times 10^7 \text{ ml}^{-1}$ in Medium 199, supplemented with 30% AB Rh⁺ human serum. Five millilitre aliquots of this cell suspension were added to 100 mm Petri dishes. Dishes were incubated at 37°C for 30 min, rocked gently for 5 min, and washed with 10 ml of M199. The rocking and washing step was repeated five times. Cells remaining adherent to the bottom of the dish at the end of the washing procedure were collected with a rubber policeman, washed, and resuspended in culture medium at a concentration of 1×10^6 cells ml^{-1} . Eighty to ninety per cent of the cells in this adherent cell preparation stained with neutral red. Identical results to those shown in the Table were obtained in four different experiments.

other species like the mouse, guinea pig, and chicken⁴⁻⁷. Our data, however, are in conflict with those of Phillips and Roitt¹, who used human B cell preparations in which at least 10% of the cells were non-B cells.

It seems that in man, as in the mouse, though pure B cells do not transform with PHA, a mixture of 5% T cells and 95% B cells exhibits a strong proliferative response to PHA which exceeds the sum of the responses of the individual T and B cell components⁸. In the mouse, karyotypic analyses of such mixtures revealed that by day 2 of culture 35% of the observed mitoses were B cells and that by day 3 of culture 97% of the mitoses were B cells. Similar data are not available for human cells.

The more-than-additive PHA response of human T and B cell mixtures could not be attributed to a soluble mediator released by T cells and acting on B cells. Human B lymphocytes, like chicken B lymphocytes, failed to proliferate in the presence of supernatants obtained from PHA-activated T cells⁷. But, in the case of *in vitro* stimulation with antigen to which cell donors are immune, it was found that the human B cell does not transform with antigen alone but transforms

TABLE 1 Characteristics of B and T lymphocyte preparations from human tonsils

Cell Source	T Lymphocytes*	B Lymphocytes†
E (+)	>95%	< 2%
EAC3 (+)	< 5%	>98-99%
Immunofluorescent (+)	< 5%	>98-100%

* E-reactive cells from fraction 4 of the BSA gradient

† E non-reactive cells from fraction 7 of the BSA gradient
Cells from fractions 4 and 7 of the BSA gradient were reacted with E or EAC3, and the lymphocyte sheep red cell mixtures were layered on top of Ficoll-Hypaque gradients and centrifuged at 400 g and 18°C for 45 min. Non-rosette-forming cells collected at the interface of the gradient, while rosette-forming cells and red cells collected at the bottom of the gradient. Cells collected from the bottom of the gradient were treated for 10 min with 0.87% NH_4Cl to lyse the red cells. Lymphocytes collected from the Ficoll gradient were washed three times in Medium 199 before culture. Cell recovery, following Ficoll gradient separation, averaged 70-80%. Viability of the recovered cells always exceeded 90%.

TABLE 3 Effect of T cell supernatants on proliferative response of human B and T cells to PHA

Addition to culture	³ H-Thymidine incorporated per culture of	
	B cells	T cells
None	c.p.m. 925	c.p.m. 812
PHA	1,067 (1.1)	82,638 (101)
24-h supernatant from T cells	877 (0.9)	763 (0.9)
24-h supernatant from T cells + PHA	1,168 (1.3)	78,314 (96.4)
24-h supernatant from PHA-activated T cells	1,309 (1.4)	98,561 (120)
48-h supernatant from PHA-activated T cells	993 (1.1)	91,426 (113)

Supernatants were derived from cultures of T cells incubated at 1×10^7 cells ml⁻¹ with or without PHA. Culture supernatants were filtered through 0.45 μ m filters (Millipore Corporation, Bedford, Massachusetts) and stored at -20° C until tested. Supernatants were added at 1:2 dilution to both B and T cells cultures. PHA concentration was adjusted to 1/100 in all cultures. Identical results were obtained in four different experiments. Values in between parentheses represent s.i.

vigorously (s.i. 20–40) in the presence of supernatants derived from antigen-activated T cells⁹. In the process, the B cell loses its C3 receptor, accumulates rough endoplasmic reticulum, and engages in antibody secretion. If the human B cell transforms with PHA only in the presence of T cells, then this could mean that intimate B cell to T cell contact is needed. It would be interesting to know if human B cells, like their murine counterparts, possess receptors for PHA as T cells but do not respond to the mitogen¹⁰ or if they lack a receptor for PHA.

It is clear from this work that it is necessary when studying the behaviour of the human B lymphocytes to reduce as much as possible contamination of the purified B cell preparations with T cells. Caution should also be exercised in extrapolating data from mouse to man, as the B cells from the two species have been recently shown to behave differently *in vitro* in response to stimulation with phytomitogens, antigens, and lipopolysaccharides (R.S.G. and E.M., unpublished).

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R. S. GEHA
F. S. ROSEN
E. MERLER

Immunology Division,
Department of Medicine
Children's Hospital Medical Center
and Department of Pediatrics,
Harvard Medical School,
Boston,
Massachusetts 02115

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Independent behaviour of blood group A- and B-like activities in gastric carcinomata of blood group AB individuals

SIMMONS and Perlmann¹ have provided evidence that the carcinoembryonic antigen (CEA) (ref. 2) is an incomplete blood group substance that lacks the type 1 chain in most of the material studied. This deficiency was explained by deletion or repression in gastrointestinal carcinomata of genes and related transferases required for the synthesis of the blood group specific macromolecules. The chains which are not incorporated apparently accumulate as suggested by the work of Hakomori *et al.*³. Since A-, B-, H-, and Le^a-specific sequences in the type 2 chain were also missing in the CEA preparations¹ Simmons and Perlmann concluded that gastrointestinal adenocarcinomata may, in addition, have more specific defects localised at the ABO, H, and Le loci. Morphological studies dealing with the distribution of blood group-like antigens in carcinomata^{4–9} have provided further proof for the disturbance of the synthesis of blood group substance in neoplasia. Davidsohn⁶ reported a correlation between the histological degree of differentiation of the tumour and the presence of blood group substance and suggested an inverse relationship between the ability of a tumour to produce blood group substance and to metastasise. In the course of morphological studies dealing with the relationship between blood group substance and CEA in tumour tissue, we studied five specimens of surgically removed gastric carcinomata from patients with the blood group A₁B and we observed independent behaviour of the A and the B specificity in four of them in certain areas of the malignant growth.

The specimens were fixed in neutral buffered formalin, embedded in paraffin and subsequently treated for the demonstration of blood group antigens by the modified mixed cell agglutination reaction (MCAR)¹⁰ as described by Davidsohn (for review see ref. 6). Commercial antisera to blood group substance (BG)-A and -B (Ortho) were concentrated to a haemagglutination titre of approximately 1:500.

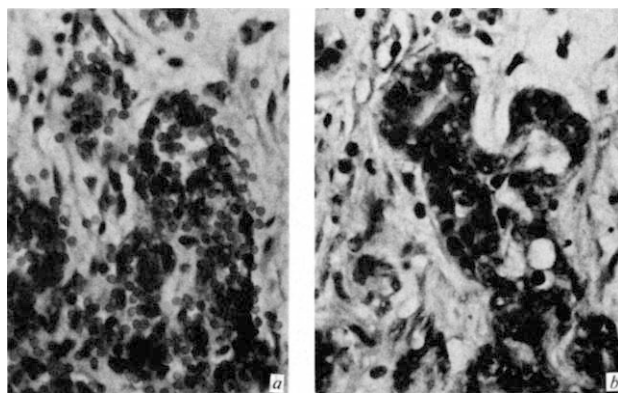


FIG. 1 Moderately differentiated gastric adenocarcinoma. a, Positive MCAR (BG-B); b, negative MCAR (BG-A). Serial sections. $\times 285$, Haematoxylin and eosin (HE).

TABLE 1 A and B blood group substance—like activity in gastric cancer tissue of patients with blood group AB

No.	11027	14283	1899	1896	2878
Histology					
Adenocarcinoma	—	A neg/B neg	A neg/B neg A neg/B neg	A neg/B pos A pos/B pos A neg/B neg	A pos/B neg
Globocellular carcinoma (anaplastic)	A pos/B pos A neg/B pos	A neg/B pos A neg/B neg	—	—	A pos/B pos
Signet ring cell carcinoma	—	—	—	A neg/B neg	A neg/B neg

Ulex europaeus extracts were used to detect H activity. According to ref. 6 blood group substances soluble in both water and alcohol are preserved during formalin fixation and paraffin embedding. After completion of the specific red cell adherence reaction the specimens were fixed in 1–5% glutaraldehyde and stained with haematoxylin-eosin. Histologically the tumours showed a variety of patterns from rather well-differentiated adenocarcinoma to anaplastic globocellular growth. Signet ring cells were present in two carcinomas. The normal superficial as well as the intestinal metaplastic mucosa were rich in both BG-A and BG-B in all of our five cases in a distribution typical for secretors^{11,12}. The endothelial cells of the blood vessels and the erythrocytes in the normal tissue as well as within the carcinoma were positive with respect to BG-A and BG-B in every case thus providing appropriate positive controls. Reagent controls were non-isologous antiserum and isologous indicator erythrocytes and *vice versa*. No relationship between the presence of blood group substances and the histological degree of differentiation was observed in our cases and different distribution patterns of BG-A and -B activities were observed even in morphologically identical areas (Table 1). No H activity was demonstrable in the tumours although control specimens of blood group O and A₂ gave positive results with the *Ulex* extract used.

CEA was postulated to result from defective synthesis of blood group substances in gastrointestinal carcinomata

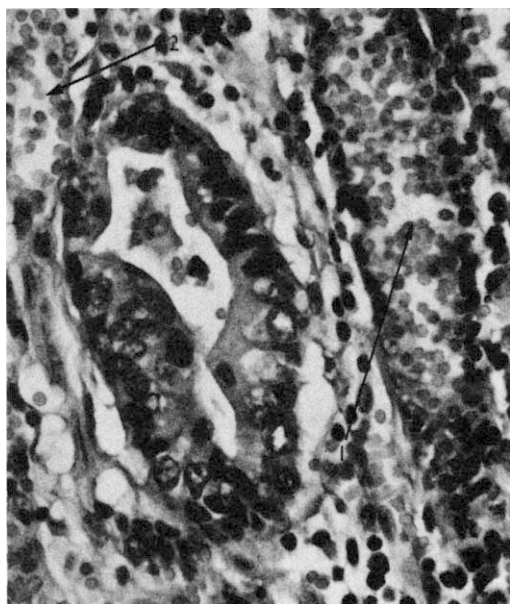


FIG. 2 Moderately differentiated gastric adenocarcinoma (same tumour as in Fig. 1). Negative MCAR for BG-B (shown) as well as for BG-A (not shown) in the tumour. Positive reaction in adjacent normal mucosa (1) and vessel (2). $\times 400$, HE.

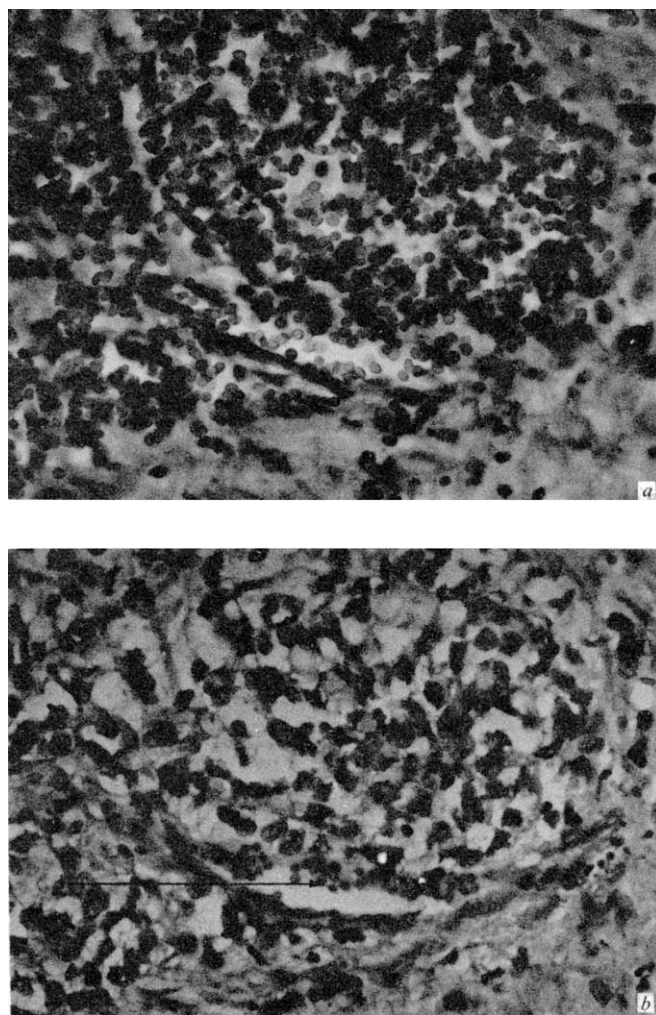


FIG. 3 Anaplastic globocellular carcinoma. *a*, Positive MCAR (BG-B); *b*, negative MCAR (BG-A). Blood group substance-positive endothelial cells (1). Serial sections. $\times 350$ HE.

due to genetic defects¹. Our results allow us to localise possible genetic defects more precisely. α -N-acetyl-D-galactosaminyl transferase and α -D-galactosyl transferase, respectively, that attach the appropriate sugars in 1 $\rightarrow$ 3 linkage to the β -galactosyl unit in an H-active structure depend upon intact A and B genes, respectively¹³. Since these transferases have identical acceptor substrate requirements¹³ the selective loss of either BG-A or BG-B is most likely to be due to deletion or repression of the corresponding gene (either A or B) in the ABO locus. A similar mechanism was discussed to explain the appearance of A₂, A₂B, B and O erythrocytes in a patient with leukaemia¹⁴. Disappearance

of both A and B blood group substances in other parts of the same carcinoma confirms that the genetic and related enzyme defects vary even in the same tumour. The exact relationship between blood group substances and CEA in cancer tissue is not yet established. Different degrees of genetically determined lesions are conceivable; selective loss of one antigenic specificity may represent the minimal change in a process that leads to the development of a CEA-specific chemical structure.

Other, less likely explanations of the selective loss of one BG specificity in A₁B patients should also be considered. One is the absence of substrates such as N-acetyl-D-galactosamine or D-galactose in the tumour. This possibility is unlikely since the intact precursor molecule relies on the presence of these carbohydrates. It is also possible that a specific A- or B-destroying enzyme exists in the tumour cells.

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H. DENK
G. TAPPEINER
J. H. HOLZNER

Department of Pathology,
School of Medicine,
University of Vienna,
Spitalgasse 4,
A-1090 Vienna

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Model for the binding of multivalent antigen to cells

It has been suggested frequently¹⁻⁴ that antigen must be presented to immunocompetent cells in multivalent or aggregated form in order to stimulate the cells to proliferate and secrete antibodies or otherwise respond. Multivalent antigens, that is antigen molecules with more than one identical epitope per molecule, seem to bind multivalently and irreversibly to the antibody-like receptors on immunocompetent cells^{5,6}, and T cells or their products may serve to aggregate antigen molecules so as to present them to other cells in multivalent forms⁷. The binding of multivalent ligands to lymphocyte receptors is known to induce receptor-ligand lattice formation and in many cases to induce mitosis and antibody secretion from B cells^{4,8,9}.

It is also known that, after exposure of an animal to antigen, the affinity of the antibodies which are produced will often increase with time^{10,11}. This maturation of the immune response could be understood^{12,13} easily if the binding of antigen to cells and cell proliferation were governed by the equilibrium constant for receptor (antibody)-epitope

binding. A small (and decreasing) concentration of free antigen would then bind cells with high (and increasing) affinity receptors and would selectively stimulate such high affinity cells. If, however, the antigen-to-cell binding is actually multivalent and irreversible⁸, it is not clear how it can be determined by the receptor-epitope equilibrium constant, and consequently how the maturation can be induced.

The purpose of this communication is to present a simple model of how the rate of multivalent and irreversible binding can be governed by an equilibrium constant for epitope-receptor binding. Although the model is highly simplified, it suggests several testable relationships between important measurable quantities.

The model is depicted in Fig. 1. Consider a mixture of multivalent antigen molecules and cells to which they can bind. In binding to a cell, an antigen molecule is assumed first to bind to a single receptor at one binding site with rate constant k_a . From this configuration the antigen molecule can either dissociate with rate constant k_d or form a bond with another receptor molecule, with rate constant k_i , thereby establishing multivalent and irreversible binding. We ignore, or alternatively, include in k_d ¹⁴, the binding of one antigen molecule to two sites on the same receptor molecule.

Let c be the concentration of free antigen, ρ and ρ_0 be the concentrations of free and total receptor sites, m be the concentration of singly bound antigen molecules, and M be the concentration of multiply bound antigen molecules, with on the average n bonds per molecule. Then the equations of the model are

$$\rho(t) = \rho_0 - m(t) - nM(t) \quad (1)$$

$$\frac{dm}{dt} = k_a c \rho - k_d m - k_i m \rho \quad (2)$$

$$\frac{dM}{dt} = k_i m \rho \quad (3)$$

Different cells may have different values of k_a , k_d , k_i , and ρ_0 . We assume that the reversible binding is near equilibrium so that as a first approximation we set $dm/dt = 0$ in equation (2) to obtain,

$$k_a c \rho = k_d m + k_i m \rho \quad (4)$$

Two limits can be considered.

(1) If $k_d \ll k_i \rho$, the dissociation rate is slow compared with the rate of establishing multivalent binding. Then from equation (4) $k_i m \rho \simeq k_a c \rho$ and substituting in equation (3)

$$\frac{dM}{dt} = k_a c \rho. \quad (5)$$

In this limit, as soon as a first bond is established subsequent bonds will be established. The rate-limiting step is first bond formation. The various antigen binding cells will differ somewhat in their values of k_a and perhaps ρ_0 , but variation in the epitope-receptor equilibrium constant, $K \equiv k_a/k_d$, is expected^{14,15} to be primarily due to variation in k_d , not in k_a . Therefore in this limit we do not have a mechanism for preferentially binding to and selecting cells based on their equilibrium binding.

(2) If $k_d \gg k_i \rho$, the dissociation rate is rapid compared with the rate to establish multiple bonds. Then equation (4) gives $m = Kc\rho$. With equation (1) this gives

$$\rho = \frac{\rho_0 - nM}{1 + Kc}, \quad m = \frac{Kc}{1 + Kc} (\rho_0 - nM) \quad (6)$$

so that equation (4) becomes

$$\frac{dM}{dt} = k_i \frac{Kc}{(1 + Kc)^2} (\rho_0 - nM)^2. \quad (7)$$

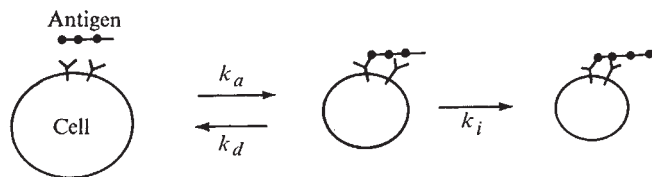


FIG. 1 Multivalent antigen is assumed first to combine reversibly with a cell receptor, with forward and reverse rate constants k_a and k_d , and then irreversible binding is established to further receptors with rate constant k_i .

In this limit, the times for both first and second bond formation are important, and the rate of establishing multivalent binding is proportional to $Kc/(1 + Kc)^2$. For cells with small values of K (that is, $K \ll 1/c$), the rate is slow because few receptors have (singly bound) antigen molecules stuck to them. If any cells have such large values of K that $K \gg 1/c$, they will also have slow rates of multivalent binding because most receptors are already singly bound to antigen molecules and therefore unavailable for establishing multiple bonds.

Equation (7) suggests a simple model for maturation of immune responses when irreversible multivalent binding of antigen is required for cell stimulation. In particular, assume that the rate of establishing multivalent binding must exceed some value, $1/T$, in order for the cells to be stimulated. Here T is a time which might be the natural lifetime of immunoglobulin receptors on cell surfaces^{16,17}. If the maximum value of the relative rate, $\rho_0^{-1} dM/dt$, must exceed $1/T$, we obtain from equation (7)

$$k_i \rho_0 \frac{Kc}{(1 + Kc)^2} > \frac{1}{T}. \quad (8)$$

Only cells with K values such that the criteria of equation (8) are satisfied would be stimulated by a free antigen concentration, c . This equation is very similar to some^{12,13} which were derived for (monovalent) equilibrium binding and which gave maturation kinetics in reasonable agreement with experiment. In these studies¹³, high zone tolerance was postulated (*ad hoc*) in that cells with large values of Kc were not stimulated and were sometimes assumed to be killed. Equation (8) clearly predicts that cells with sufficiently high values of Kc will not be stimulated.

Equation (7) can be solved readily. If antigen is first introduced at $t = 0$ and c is thereafter constant, the solution is

$$M = \left[\frac{\rho_0}{n} \frac{\alpha \rho_0 t}{1 + \alpha \rho_0 t} \right] \quad (9)$$

where $\alpha \equiv n k_i K c / (1 + K c)^2$.

Which limit is more likely to apply? For the dissociation of haptens or small antigens from antibodies, k_d is typically¹⁵ $1-100 \text{ s}^{-1}$, so that we assume that for antigen bound to a receptor at one site, typically $k_d \gtrsim 1 \text{ s}^{-1}$. For those antigens that can rapidly establish a double bond to one receptor molecule, k_d is much smaller, perhaps typically¹⁴ $\sim 10^{-3} \text{ s}^{-1}$. The rate $k_i \rho_0$ to establish a bond to a second receptor molecule will depend on the diffusion of receptors in the membrane and has been estimated to be¹⁸

$$k_i \rho_0 = a n t_D^{-1} \equiv a n \frac{D}{d^2} \quad (10)$$

where d is the mean spacing between receptor molecules ($d \simeq 40 \text{ nm}$ for B cells¹⁸), D is the receptor diffusion coefficient¹⁹ ($D \gtrsim 5 \times 10^{-11} \text{ cm}^2 \text{ s}^{-1}$), $n t_D^{-1}$ is a diffusion limit to the reaction rate and a is the factor by which the actual rate differs from the diffusion limit. For $a n \simeq 0.01$, $k_i \rho_0 \gtrsim 0.03 \text{ s}^{-1}$. We therefore conclude that for antigens which bind to only one site on a receptor molecule, limit

(2) and equation (7) may apply, while for antigens which bind easily to two sites on a receptor molecule, limit (1) and equation (5) probably apply. The precise configuration of epitope binding sites on receptors is not known and it is possible that for some kinds of cells, only one site per receptor molecule may be accessible for binding antigen. It is known that contact with antigen can modulate the number of cell receptors, but such refinements are ignored in the present model.

The model suggests that to achieve response maturation together with multivalent binding, the condition

$$k_d > a n \frac{D}{d^2} \quad (11)$$

should be satisfied. Maturation is therefore favoured by (1) large k_d , that is, rapid release of initially bound antigen such as is expected for binding to a single site on the receptor molecule, (2) small D , that is, sluggish diffusion of receptor molecules, (3) large d , that is, large spacing and small density of receptors, and (4) small antigen valence, n . These three first factors may differ between various cell classes, such as T and B cells, or between different stages in cell maturation. There is some evidence²⁰ that cells in an IgM response may not mature whereas those in an IgG response do mature. If so, the model suggests that the cells which are proliferating in IgM(IgG) responses do not (do) meet equation (11). During maturation, a clone might switch from IgM to IgG production and begin to meet equation (11) by changing the number, mobility or accessibility of its receptors.

This model indicates some simple ways in which receptor properties may affect the kinetics of antigen binding to cells and cell behaviour. It also suggests the importance of quantitative measurements of receptor number and mobility and of antigen-to-cell binding kinetics in elucidating the mechanisms which operate in antigen selection of clones, either for amplification or suppression.

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GEORGE I. BELL

University of California,
Los Alamos Scientific Laboratory,
Los Alamos, New Mexico 87544

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Localisation of sites of GABA catabolism in the rat retina

GAMMA aminobutyric acid (GABA) has been demonstrated in invertebrate and vertebrate retinæ^{1,2,3} and has been shown to have an inhibitory action on retinal neurones⁴. *In vivo* studies of ³H-GABA uptake by rabbit retina have shown a distribution of radioactivity in the inner plexiform layer, the amacrine cells of the inner nuclear layer, the ganglion cell and nerve fibre layers⁵. Further studies have shown that all layers of the rat retina except the outer zone of the rods will accumulate ³H-GABA *in vitro*⁶ in the presence of amino-oxyacetic acid (AOAA), an inhibitor of GABA metabolism⁷. Uptake was most pronounced in the ganglion cell and nerve fibre layer, inner nuclear and outer plexiform layers and at the outer limiting membrane. Furthermore, it was suggested that ³H-GABA might be largely taken up by Müller's fibres and their nuclei rather than by amacrine cells in the inner nuclear layer of the retina.

Other reports have described sites of enzyme activities involved in GABA metabolism in the retina. Glutamate decarboxylase (GAD) was unevenly distributed across the six well-defined layers of the dark-adapted frog retina⁸ and in layers of the rabbit retina¹. GABA-transaminase (GABA-T) was present in the frog retina in both the light and dark adapted states² although attempts to localise GABA-T at the cellular level in the rabbit retina were unsuccessful¹.

Previous studies have shown the ability of retinal cells to accumulate ³H-GABA, the presence of a GABA-synthesising enzyme (GAD) and correlative amounts of its product in retinal cells³. If GABA were involved in inhibitory transmission in the retina⁴, there would be a requirement for a GABA-degradatory process. The present histochemical study investigates the localisation of the GABA-degrading enzyme GABA-T in the rat retina.

Intact eyes from adult (250–300 g) Sprague-Dawley rats were removed under ether anaesthesia, embedded in supporting tissue on microtome chucks and quenched in liquid nitrogen; preliminary studies indicated that there was no contamination from the embedding tissue. The chucks were placed in a cryostat compartment and after equilibrium at -18°C , unfixed sections were cut at $12\text{ }\mu\text{m}$, mounted onto glass slides and freeze dried over activated silica gel at -20°C for a minimum of 3 h. GABA-T activity was demonstrated by a previously described dehydrogenase/tetrazolium-coupled system^{8,9}. In control studies tissue sections incubated with all components except GABA produced a very faint reaction only after extended incubation, which was probably due to NADH₂ diaphorase, an integral part of any tetrazolium-linked system. Sections incubated without α -ketoglutarate also showed a very weak reaction after extended incubation but an intense well-localised formazan precipitate was only seen when all substrate components of the incubation medium (GABA, α -ketoglutarate, NAD⁺) were included. The specificity of this method has been described elsewhere⁸. Routine Ehrlich's haematoxylin and eosin stains were carried out on alternate sections in order to identify the retinal layers.

Light microscopic examination after 30 min incubation revealed that a reaction was mainly present in the ganglion cell and inner plexiform layers, the inner nuclear and photoreceptor layers and the pigment cell layer. The reaction was absent in cells of the outer nuclear layer and in the outer and inner limiting membranes (Fig. 1a). The moderate reaction in the cytoplasm of the ganglion cells of the ganglion cell layer was similar to the reaction seen in the neuroglia in this layer. The strong reaction in the inner plexiform layer corresponded to the site of synapses between bipolar and amacrine cells and the ganglion cells. Some cells of the inner nuclear layer exhibited a good reaction, however, comparison of this stained layer with an H and E stain (Fig. 1b) showed that not all cells in the inner nuclear layer showed the enzyme

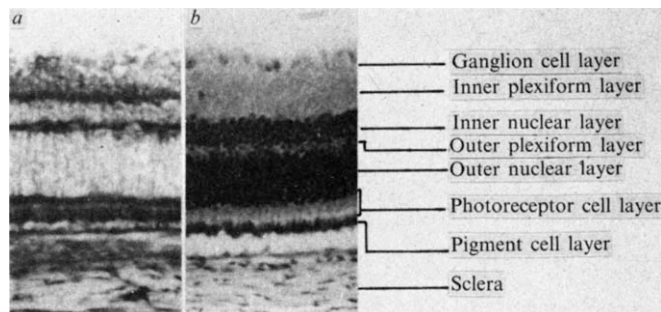


Fig. 1(a), Sites of the GABA-degrading enzymes GABA-T in transverse section of rat retina. Note reaction in the ganglion cell layer, inner plexiform layer, inner nuclear layer and photoreceptor layer (X357). (b), Transverse section of rat retina. Ehrlich's haematoxylin and eosin stain (X357)

activity. A reaction in cells of the outer zone, comprising mostly of horizontal cells, was absent. The reaction was also absent in the bipolar cells of the outer intermediate zone. However, a strong reaction was seen in the zones containing the cell bodies of Müller's fibres (inner intermediate zone) and the amacrine cells (inner zone) (Fig. 2). A reaction was seen in the sustentacular fibres of Müller which extended through the retinal layers between the outer limiting membrane and the ganglion cell layer; the reaction was clearly defined where the fibres terminated in large conical expansions at the vitreal aspect (Fig. 2).

No reaction was seen in the outer plexiform layer at the site of synapses between the rod and cone cells and the bipolar cells. An intense reaction was seen in the inner zone of the photoreceptor layer outside the outer limiting membrane, and in a thin layer of rod and cone cell bodies immediately adjacent to the vitreal aspect of the outer limiting membrane. The reaction was weak in the outer zone of the photoreceptor layer. The epithelial cells of the pigment cell layer and cells of the sclera exhibited a moderate reaction.

Information has accumulated to indicate that the retina possesses a high affinity active uptake system for GABA^{10,11}. It has been suggested that besides uptake and storage of ³H-GABA by 'GABA-neurones'¹⁰ this system also exists for the removal of GABA from retinal neurones or the neuronal environment after release from inhibitory nerve endings¹¹. Other data indicate that the Müller gliocytes of the retina may be largely responsible for inactivation of GABA by this uptake process^{3,6}. We propose that Müller gliocytes are also responsible for the degradation of GABA, which occurs in these cells after GABA uptake and is indicated by high levels of GABA-T activity in Müller cell bodies and fibres running radially throughout the retina. In support of this, intravitreal injection of ³H-GABA failed to produce labelling of Müller cells *in vivo* 4 to 12 h after injection⁵, probably due to catabolism of GABA by the high GABA-T activity now demonstrated in Müller's cells. Inhibition of retinal GABA-T by AOAA however potentiates accumulation of ³H-GABA in the retina⁷, especially in Müller's cells⁶. This strongly suggests that these glial cells are partly responsible for the uptake and degradation of GABA in the retina and that GABA is not stored after *in vivo* uptake into these cells.

Although amacrine cells possess high levels of GABA-T activity they also accumulate ³H-GABA *in vivo*^{5,10} unlike Müller gliocytes. This suggests that a protective store for GABA exists in the amacrine cells; a protective store for GABA is believed to occur in some retinal neurones¹². In this way, GABA is stored for liberation during neuronal activity, possibly during inhibitory action on ganglion cells (for refs see ref. 10). The presence of GABA-T in amacrine cells may act as a limiting factor on GABA storage, although the process for this is obscure and cannot be shown by this study.

An uneven distribution of endogenous GABA, ^3H -GABA uptake, GAD activity^{1,3,5,6,10} and GABA-T activity in the retina has been demonstrated. Within the inner plexiform layer there are high levels of GAD and GABA-T activity. GAD activity is thought to be located predominantly in amacrine nerve endings³ suggesting that the amacrine may be the main GABA synthesising cells of the retina. From present data, GABA-T activity is thought to be localised essentially in the meshwork of Müller's fibres and associated fibrillae which surround the synapses of the inner plexiform layer and not in the synapses themselves. Light microscopy cannot completely resolve this feature but the conclusion is supported by the observation that GABA-T activity in Müller's fibres increases in areas corresponding to synaptic regions, where the fibres pass through the inner plexiform layer.

The presence of high levels of GABA-T activity in the inner zones of the photoreceptor layer and in a thin layer of rod and cone cell bodies at the outer limiting membrane is also intriguing. These zones have very low GAD activity and low levels of endogenous GABA³; they accumulate ^3H -GABA *in vitro* in the presence of AOAA but not *in vivo*^{5,10}. This indicates that GABA-T is probably involved in GABA degradation in these zones. GABA-T activity may be present in the rod and cone cells but the uneven distribution of GABA-T activity and ^3H -GABA uptake indicates that there are specialised areas or structures involved. Fibrillar baskets originating from Müller's fibres at the external limiting membrane form around the proximal parts of the photoreceptor layer and it is possible that these baskets are the main sites of GABA-T activity and ^3H -GABA uptake *in vitro*⁶. The presence of a GABA-T mediated GABA inactivating mechanism in the photoreceptor layer would support

recent evidence that horizontal cells, which synapse with rod and cone cells, have a GABA-related inhibitory function in the retina^{3,13}. The uneven distribution of the GABA system within the retinal layers indicates that the system is compartmentalised between retinal cells. The evidence suggests that GABA synthesis, uptake and storage occurs in neuronal compartments, whilst uptake and degradation of GABA takes place to a large extent in a separate cellular compartment, probably glial. This is in agreement with other investigations into the GABA system in nervous tissue^{3,5,6,10,14,16}.

To obtain further information on the localisation of GABA-T in the inner plexiform and photoreceptor layers, we are currently examining the ultrastructural localisation of GABA-T activity in these areas.

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J. C. HYDE
N. ROBINSON

Department of Anatomy and Histology,
The London Hospital Medical College,
Turner Street, London, E1 2AD

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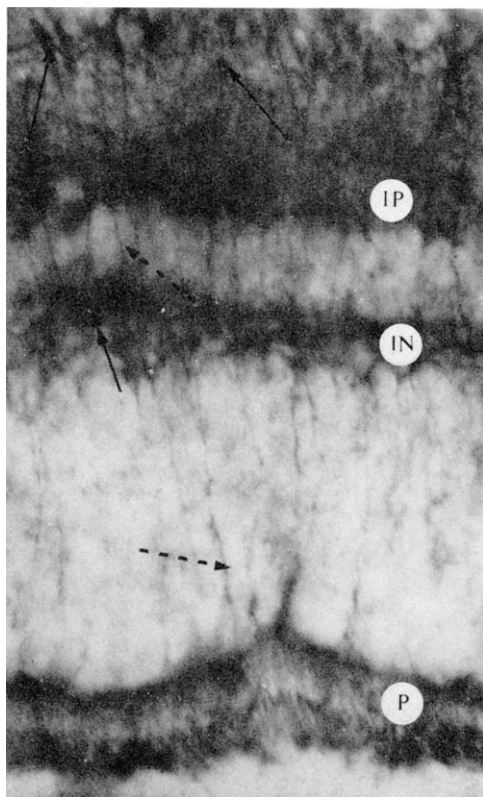


Fig. 2 Sites of the GABA-degrading enzyme GABA-T in transverse section of rat retina. Note strong reaction in Müller's fibres (broken arrows), their cell bodies (double headed arrow) and the conical expansions at the vitreal aspect of the fibres (single headed arrows). A reaction is also present in the inner plexiform layer (IP) and photoreceptor layer (P) and the inner nuclear layer (IN) (x1,750).

Remote nerve fibre bundle alterations in the retina as caused by argon laser photocoagulation

CONSIDERABLE information has been accumulated on the morphological characteristics of retinal lesions produced by laser sources¹⁻⁴. The argon laser is the most widely used energy source for clinical photocoagulation because of its optical and operational qualities.

Laser-induced retinal alterations characterised so far indicate that with increased energy, damaged areas include outer segment layers as well as the pigment epithelium, which is considered the primary site of absorption^{2,3}. Extremely low levels of coherent radiation also produce ultrastructural alterations in sensory retina without apparent change in pigment epithelium⁵. Most reports however have dealt primarily with the lesions or successful treatment⁶⁻⁸. Little information is available on possible secondary sites of injury which may not be spatially or temporally contiguous with the primary lesion. We describe here secondary alterations found considerable distances from the site of primary impact and not occurring immediately.

Single clinical level exposures were placed on retinal vessels as well as vessel-free areas in twelve rhesus monkey (*Macaca mulatta*) eyes. An argon laser, operating at 514.5

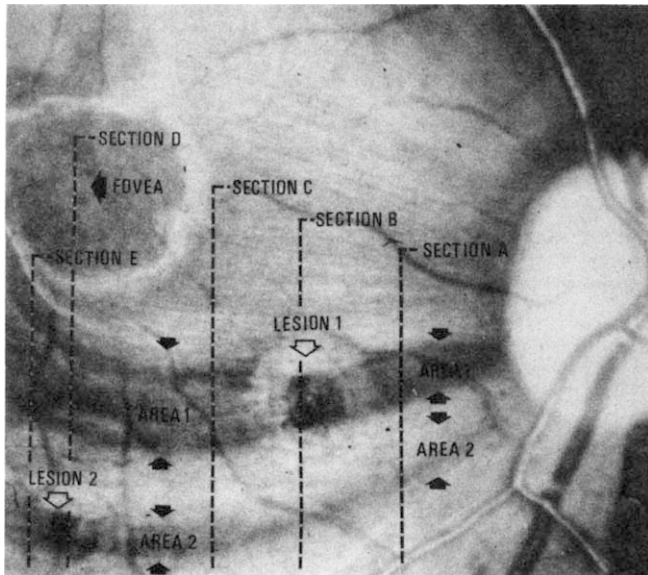


FIG. 1 Fundus photograph of 10-week-old exposures. Lesion 1 was placed at 300 mW, 1 s exposure and 150 μ m spot size. Lesion 2 was placed at 160 mW, 1 s exposure and 150 μ m spot size.

nm and TEM₀₀ mode, was used. The animals were observed for up to 1 year after exposure using direct ophthalmoscopy and fundus photography. Six eyes, from three animals, were enucleated and serial sections, stained with haematoxylin and eosin, were studied under light microscopy. Exposure levels, pulse durations and spot sizes used are given in Table 1.

Immediately after exposure, areas peripheral to the lesions exhibited no ophthalmoscopically visible changes. The colour and capillary network of the optic disk and surrounding tissue appeared normal. Within 5–10 d, however, a darker

area of tissue, of arcuate course and running through the lesions, extended both centrally toward the disk and peripherally away from the lesions, apparently following the distribution of nerve fibres and persisting for 1 yr after exposure (Fig. 1).

Examination of histo-pathological sections, taken 10 weeks after exposure, confirmed the presence of alterations corresponding to the darkened areas. Centrally toward the disk, a diminution of nerve fibres was evident. Other layers of the retina seemed to be unaltered (Fig. 3a). In the area of the lesions, outer segment, inner segment and outer nuclear layers of the retina as well as the pigment epithelium were affected, the extent of damage diminishing with distance from the centre of the impact site (Fig. 3b). Peripheral to the lesion sites, the thinning of the nerve fibre layer was accompanied by a diminution of ganglion cells (Fig. 3c). Again, as was the case central to the lesion all other retinal layers appeared unaltered. This effect is amply demonstrated in Fig. 2, corresponding to the fundus photograph (Fig. 1). Section A is central to both lesions, and compression of the nerve fibre layer, corresponding to the two darkened areas, is evident. Section B is through the site of lesion 1. Section C is peripheral to lesion 1 and central to lesion 2. The effect of lesion 1 shows compression of nerve fibres as well as thinning in the ganglion cell layer, while the effect of lesion 2 remains relatively unchanged from section A. Section D shows lesion 2 and the fovea. Section E is peripheral to both lesion sites and demonstrates the compression of nerve fibres and loss of ganglion cells in both cases. This effect seemed to be more pronounced in lesions placed on vessels than those placed on vessel-free areas.

The implications of this finding are numerous. Since damage to the nerve fibre bundle seems to be involved, the retina is being altered far from the primary site of damage. It has been reported that whenever there are visible nerve fibre defects in the arcuate or radial nerve fibres, corresponding defects are found in the visual field⁹. This type of damage implies both retrograde axonal, as

FIG. 2 Serial sections corresponding to Fig. 1. Section A is central to both lesions. Section B shows lesion 1 and thinning of nerve fibres corresponding to lesion 2. Section C depicts areas peripheral to lesion 1 and central to lesion 2. Section D shows lesion 2 and diminution of nerve fibres and ganglion cells as a result of lesion 1. Section E shows peripheral areas to both lesions 1 and 2.

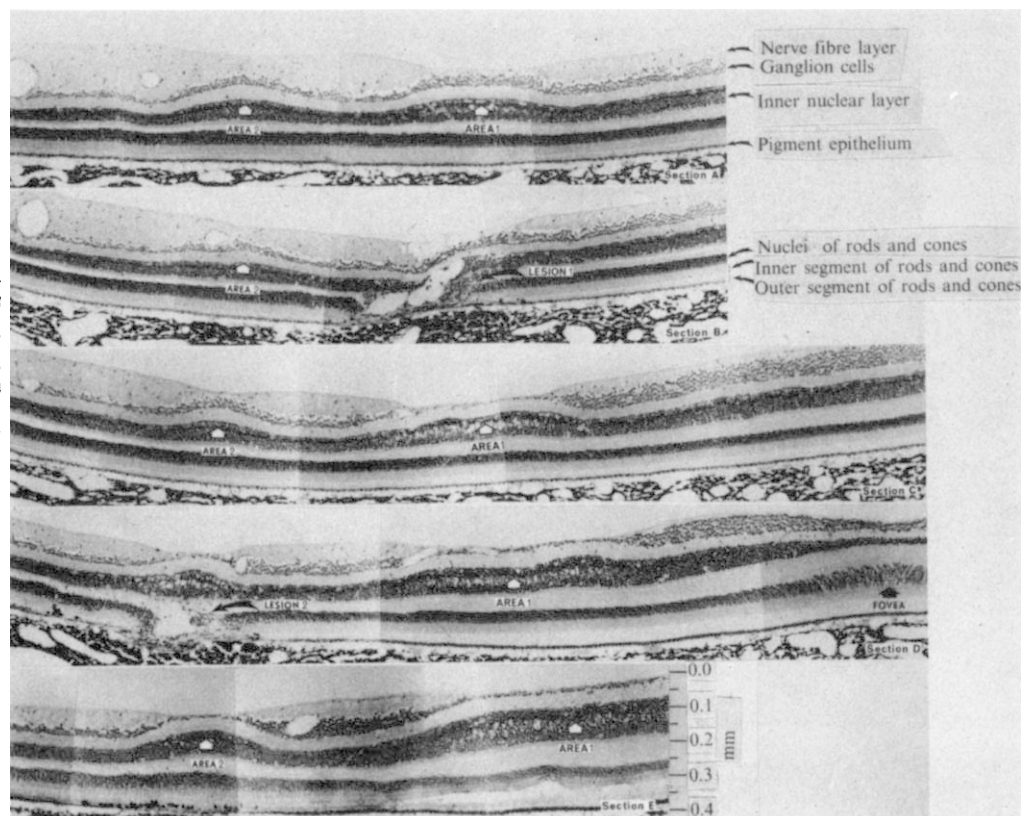


TABLE 1 Power Levels, Spot Sizes, Pulse Durations and Retinal Locations used in obtaining Ophthalmoscopically Visible Nerve Fibre Damage

Power level (mW)	Spot size (μm)	Pulse duration (s)	Retinal location	Ophthalmoscopically visible Nerve fibre damage
125	100	1.00	Retinal vessel	1 of 1
			Vessel-free area	1 of 1
125	500	1.00	Retinal vessel	2 of 4
			Vessel-free area	0 of 1
160	150	1.00	Retinal vessel	1 of 1
300	150	1.00	Retinal vessel	1 of 1
250	50	0.50	Retinal vessel	0 of 1
350	50	0.50	Retinal vessel	1 of 1
250	500	0.25	Retinal vessel	2 of 2
			Vessel-free area	1 of 1
180	150	0.25	Retinal vessel	0 of 1
200	100	0.25	Retinal vessel	2 of 2
			Vessel-free area	0 of 1

well as Wallerian or ascending degeneration. One can generalise that the presence of these ophthalmoscopically visible dark streaks would indicate permanent involvement of optic axons and that the size of the field defect will equal or exceed the area predicted from the size of the retinal nerve fibre bundle damage.

Clinically, nerve fibre field defects have been associated with extremely high retinal energy densities and with repeated photocoagulation about the optic disk¹⁰. Seldom have they been assumed to be associated with initial treatment at moderate power levels. Presumably, the damage is thermal, where the increase in temperature is attributed to the radiation of heat from the underlying pigment epithelium and converted into heat. After the initial photocoagulation, the retina is assumed to become thin so that the nerve fibre layer is in closer proximity to the retinal pigment epithelium causing a greater thermal gradient than in the initial treatment. Clinical power levels range from approximately 300 mW. (2 s pulse, 50–100 μm spot size) used to treat histoplasmosis choroiditis, to 100 mW. (0.1 s pulse, 50 μm spot size) used to treat retinal neovascularisation. Clearly our exposures lie within this range. Since rhesus monkey retina would tend to be more sensitive to laser radiation than that of humans (assuming the previously

discussed thermal considerations to be true), it is conceivable that this demonstrated effect is not as pronounced in the latter. This finding however does indicate that nerve fibre bundle damage is a distinct possibility at the presently used power levels. Much more work is required to establish the relationship of energy density and pulse duration to nerve fibre damage.

GEORG D. FRISCH
PAUL D. SHAWALUK
DOLPH O. ADAMS

Joint AMRDC-AMC Laser Safety Team,
Frankford Arsenal,
Philadelphia, Pennsylvania 19137

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Temporal pattern of placental lactogen and progesterone secretion in sheep

HUMAN and monkey placental lactogens (PL) have been isolated and characterised biologically and chemically¹⁻³ and reports have appeared identifying a placental lactogen in rats⁴⁻⁶, mice⁷ and goats⁸. More recently rat serum PL concentrations have been determined quantitatively with a radioreceptor assay⁹. In the present study we have used the same assay with minor modifications to identify and measure plasma placental lactogen concentrations in sheep during pregnancy and have compared the secretory pattern of this new hormone with plasma concentrations of progesterone and pituitary prolactin.

We assayed plasma samples obtained at weekly intervals throughout pregnancy in three sheep. Blood samples were collected from animals housed in individual pens by needle puncture from the jugular vein into heparinised tubes. After centrifugation plasma samples were kept frozen at -20°C and some were stored for as long as 2 yr before assay. Lactogenic hormone in these samples was assayed by a radioreceptor assay which has been shown to be specific for lactogenic hormones. The assay uses membranes prepared from mammary glands⁹ of term pregnant rabbits. Plasma concentrations of pituitary prolactin were measured by a homologous, double antibody radioimmunoassay for ovine prolactin (OPRL) using ovine prolactin (NIH-P-S10, 26 IU mg^{-1}) as standard¹⁰. The progesterone concentration in these samples was determined by competitive protein binding assay¹¹.

We also examined in more detail the time course of changes in the concentrations of the three hormones that occur at parturition. Pregnant sheep were bled at intervals of 6 h from 96 h before to 60 h after parturition and the concentrations of all three hormones were determined.

Particulate membrane receptors were incubated with ¹²⁵I-OPRL and displaced by increasing concentrations of

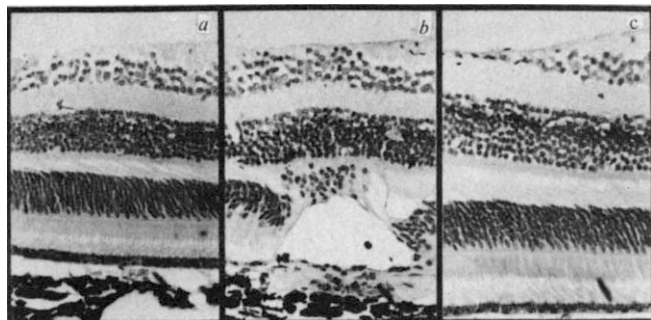


FIG. 3 *a*, Serial section of area central toward disk. Note the diminution of nerve fibre layer. (200 mW, 100 μm spot size and 0.25 s pulse). *b*, Peripheral section of impact site. Note the thinning of nerve fibres and involvement of pigment epithelium, outer segment, inner segment, and outer nuclear layers. *c*, Area peripherally away from lesion. Note absence of nerve fibre layer and diminution of ganglion cells. Other layers of retina seem to be unaffected.

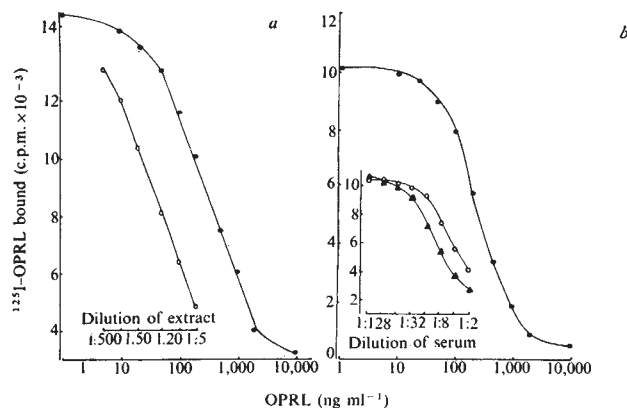


Fig. 1 *a*, Displacement curves obtained when particulate membrane receptors were incubated with ^{125}I -OPRL (100,000 c.p.m.) in the presence of increasing concentrations of OPRL or dilutions of ovine placental extracts. The ordinate represents the amount of ^{125}I -OPRL bound to the receptors expressed as c.p.m. and the abscissa is the concentration of OPRL added to the assay tube. Values for tube blanks were subtracted from the total ^{125}I -OPRL bound to the membranes. The tube blanks were determined by counting the tubes which contained no membranes and were usually 3–4% of the total radioactivity added. The incubation was carried out at room temperature for 6 h. Membrane-bound and free hormone were separated by low speed centrifugation (750*g*) for 30 min. Foetal cotyledons were extracted for 4 h in 0.1 M NH_4NCO_2 (1 g per 20 ml) centrifuged and dilutions of the extract were assayed. *b*, Displacement curves obtained with serum or plasma samples. The assay procedures were identical to those described in the legend to (*a*), except that 25 μl of either normal human male serum (NHMS), hypophysectomised rat serum or normal sheep serum were added to each of the tubes containing the OPRL standards. When plasma rather than serum samples were assayed, it was necessary to add heparin to the diluting buffer to inhibit clot formation. The inset shows inhibition curves of dilutions of day 114 pregnant sheep plasma and OPRL in the presence of equivalent dilutions of NHMS. The 1:2 dilution was prepared by combining equal quantities of NHMS and OPRL, 2 $\mu\text{g ml}^{-1}$. *a*: ●, OPRL standard; ○, ovine placental extract. *b*: ●, OPRL standard (+ serum); ○, dilutions of day 114 pregnant sheep plasma; ▲, OPRL in presence of dilutions of NHMS.

unlabelled OPRL (Fig. 1). The curves obtained with dilutions of ovine placental extract were parallel to the OPRL inhibition curves (Fig. 1*a*). Despite concentrations of lactogen greater than 8 $\mu\text{g ml}^{-1}$ in the placental extract, the radioimmunoassay for pituitary prolactin failed to detect any lactogen in these extracts. The displacement curve for serum or plasma assays is shown in Fig. 1*b*. In this assay procedure the prolactin standard contained 25 μl of normal human male serum (NHMS). The inset shows the parallelism of dilutions of day 114 pregnant sheep plasma and OPRL in the presence of equivalent dilutions of NHMS.

Figure 2 shows the plasma concentrations of PL, prolactin and progesterone in samples from three ewes. Placental lactogen can usually be detected in plasma samples by day 60 of gestation and thereafter it increases as pregnancy advances, reaching peak concentrations of 1,000 to 2,000 ng ml^{-1} on days 95 to 114 of gestation. After the initial peak there is generally a decline in PL concentration followed by one or more peaks before parturition. These fluctuations, especially in ewe 187 (Fig. 2*c*) are reproducible and not due to assay variation, as similar overall patterns were observed on re-assay of the same plasma samples. The plasma concentrations of pituitary prolactin measured by radioimmunoassay remained below 50 ng ml^{-1} until a few days before parturition. So, except at term, the concentrations of PL measured by radioreceptor assay in plasma samples of pregnant sheep cannot be ascribed to the concentrations of prolactin of pituitary origin which is in the circulation.

Progesterone concentrations in two of the three animals very closely paralleled the temporal pattern of PL concentrations. In ewe 187 during the second half of pregnancy several peaks of PL were noted, only one of which exceeded 1,000 ng ml^{-1} . In spite of this, the progesterone concentrations in this animal were similar to those of the other two ewes.

The plasma concentrations of PL, prolactin, and progesterone at the time of parturition in three sheep are shown in Fig. 3. Placental lactogen concentrations slowly declined from approximately 1,000 ng ml^{-1} to 500–700 ng ml^{-1} by 12 h before parturition; they then decreased quite rapidly *post partum*. Plasma concentrations of pituitary prolactin began to rise about 36 h before parturition, reaching levels of 300–600 ng ml^{-1} approximately 3–6 h before parturition. At this time the PL concentrations measured by radioreceptor assay were almost entirely due to the cross reaction of pituitary prolactin in the receptor assay. During this time progesterone concentrations also fell rapidly together with PL concentration. In two of the three sheep the decrease in progesterone preceded that in PL by 6–12 h.

To estimate the relative half-time rate of disappearance of PL, the uterine vessels of two ewes were ligated on day 140 of pregnancy and plasma samples were collected. Assay of these samples by radioreceptor assay showed that PL disappeared from the circulation with a half-time of less than 20 min.

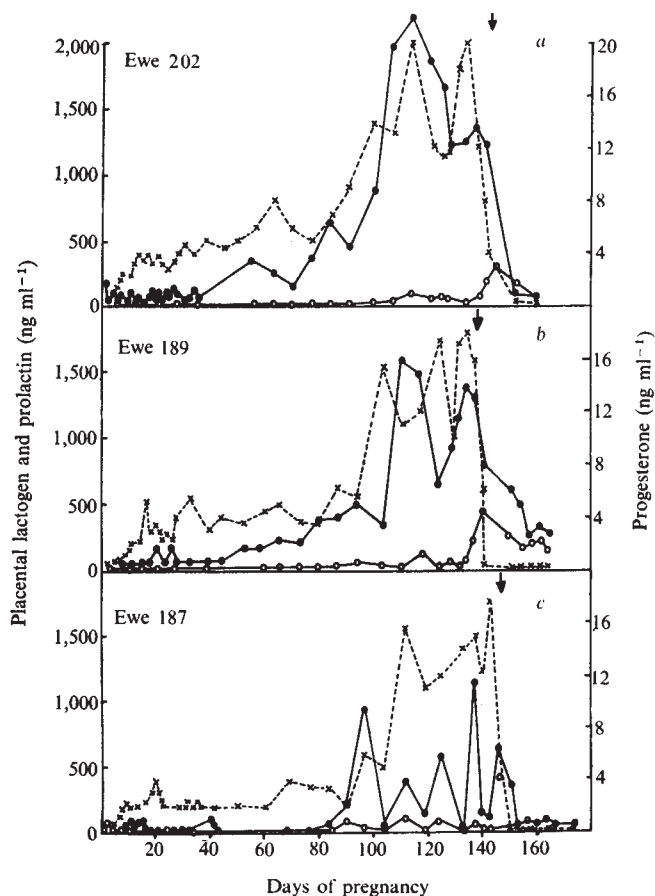


Fig. 2 Plasma concentrations of placental lactogen (PL) were measured by radioreceptor assay, plasma concentrations of pituitary prolactin (PRL) were measured by radioimmunoassay, and progesterone levels were determined by competitive protein binding assay during pregnancy in three sheep. The radioreceptor assay detects both PL and pituitary prolactin, however, except at term, the plasma concentration of the latter is trivial by comparison with PL levels. The radioimmunoassay for pituitary prolactin fails to detect placental lactogen. The arrow (↓) indicates the day of parturition. ●, Placental lactogen; ×, progesterone; ○, prolactin.

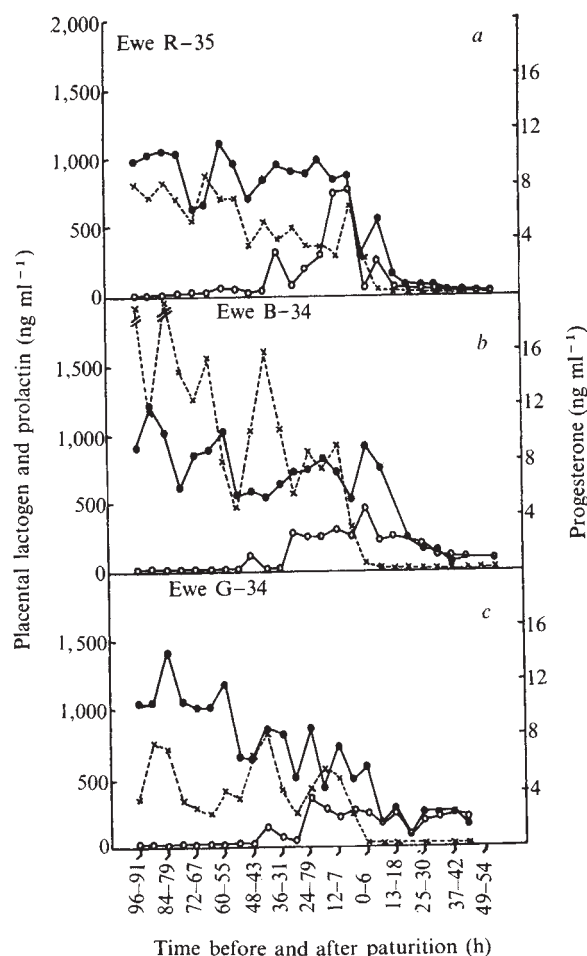


Fig. 3 Placental lactogen, prolactin and progesterone levels from 96 h before to 60 h after parturition in three sheep. Parturition occurred at some time between the intervals 6-0 and 0-6 h. ●, Placental lactogen; ×, progesterone; ○, prolactin.

Both the foetal and maternal cotyledons were extracted in 0.1 M NH_4HCO_3 . The concentration of PL in both extracts was 200 μg per wet weight of tissue which is similar to the concentration of PL human placental extracts². After fractionation of ovine placental extracts on Sephadex G-100, PL appeared in the same elution volume as ^{125}I -OPRL which had been added as a marker, suggesting that the molecular weights of PL and pituitary prolactin are very similar.

Several hormonal changes have been documented at the time of parturition in sheep. These include sequentially, an increase in blood cortisol in the foetus¹², followed by an increase in total unconjugated oestrogens¹³ and in the individual unconjugated oestrogens¹⁴ and a decrease in serum progesterone in maternal blood¹⁵ and, as we show here, a parallel decline in PL concentrations. The precise interrelationship and significance of all these hormonal changes is still obscure. Nevertheless, the general similarity of the temporal pattern of plasma concentrations of PL and progesterone is sufficiently intriguing to suggest that PL may stimulate progesterone production. With the purification of ovine PL in adequate amounts it should be possible to test this hypothesis directly. The fact remains that a new PL has been identified and quantified in plasma and placental extracts of pregnant sheep and this observation offers a new model to study the elusive role of placental lactogen.

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PAUL A. KELLY*

McGill University Clinic,
Royal Victoria Hospital,
Montreal 112, Quebec

HAMISH A. ROBERTSON

Reproductive Physiology,
Animal Research Institute,
Ottawa, Ontario K1A 0C6

HENRY G. FRIESEN*

McGill University Clinic

Received October 1; revised December 4, 1973.

* Present address: Department of Physiology, University of Manitoba, Faculty of Medicine, Winnipeg, Manitoba.

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Cortical hypothermia is a sequela of electroconvulsive shock

THE impaired retention of a recently learned habit that is commonly observed in experimental animals after administration of electroconvulsive shock (ECS) is also observed after partial immersion for a few minutes in cold water¹⁻⁴. The physical differences between a hypothermal and an electrical amnesic agent do not preclude a common mode of action; indeed, we have confirmed that ECS results in hypothermia as well as amnesia.

Eight mature, partially pigmented rats were implanted surgically, under barbiturate anaesthesia, with two 2.5 mm stainless steel screw electrodes that were used to administer ECS and also to record the electrocorticogram (ECoG). In each rat electrodes were threaded through small bur holes in the calvarium to contact the dural membrane about 5 mm lateral to the midline, one electrode to the frontal cortex and one to the contralateral occipital cortex. A third electrode was screwed into the calvarium at the midline about 10 mm anterior to the bregma suture; this later served as an electrically indifferent pole for recording the ECoG. Each rat was also fitted with a small thermistor (United Systems model 430) that was lowered through a bur hole in the calvarium and then through an incision of the meninges to rest on parietal cortex near the vertex. Insulated conductive leads from each of the screw electrodes, and from the two leads of the thermistor, were dressed closely to the calvarium and then individually terminated at one of the five connector pins of a small socket anchored to the calvarium by dental cement. For a minimum of 7 d after surgery, the rats were housed continuously in individual cages in a vivarium where the ambient temperature was maintained between 22° and 26°C,

and relative humidity was between 50 and 70%. Purina Lab Chow was available *ad lib* in the home cage; so too was drinking water, until commencement of pretraining.

TABLE 1 Numbers and means of entries of ten control and eight experimental rats during formal training trial of the tenth day and during the retention trial of the twelfth day.

	Control			Experimental		
	Pre-ECS*	Post-ECS†	Diff.‡	Pre-ECS*	Post-ECS†	Diff.‡
	17	28	11	12	11	-1
	9	21	12	12	9	-3
	7	17	10	10	19	9
	6	21	15	9	13	4
	5	21	16	6	14	8
	5	20	15	5	7	2
	5	13	8	3	4	1
	4	32	28	3	4	1
	3	24	21			
	2	18	16			
<i>M</i> =	6.3	21.5	15.2	7.5	10.1	2.6
	$P < 0.001$			$P > 0.05$		
				$P < 0.001$		

* Formal training trial of tenth day.

† Retention trial of twelfth day.

‡ Difference between number of Post-ECS and Pre-ECS entries.

Our measure of amnesia was a one-trial appetitive learning task developed by Tenen⁵ and independently confirmed^{6,7} as a mnemonic assay that controls for possible aversive sequelae of ECS. The task initially involved 10 successive days of pretraining during each of which, after 22 to 24 h without water, a rat was permitted to explore for 5 min the interior of an open-topped enclosure containing a nook in one wall. During all trials of pretraining, formal training and testing for retention, a set of flexible conductive leads was fastened to a rat's calvarium-pedestal; the shape of the nook accommodated the leads in such a way that an animal could enter and leave it without restriction. The formal training trial was conducted during the eleventh day, the only day in which a drinking tube that provided water was present in the nook. Fifteen seconds after each animal had begun to drink it was removed from the enclosure and was held while 40 mA (root-mean-square) of 60 Hz alternating sine-wave current was passed between the frontal and occipital screw electrodes for 0.5 s. Ten control rats, which shared a common history of origin, housing, surgery and pretraining, received sham-ECS treatment—no electrical current—but were otherwise handled in the same way. During the twelfth day, 22 to 24 h after ECS or sham ECS, each of the eighteen rats was returned to the enclosure and observed for 5 min, as under pretraining conditions, and its entries into the nook were counted.

The averaged number of entries of the ten unshocked rats of the sham-ECS group increased more than three-fold between the tenth day (6.3) and the twelfth day (21.5); on the twelfth day they behaved as if they expected to find water. In contrast, the eight shocked animals performed during the twelfth day about the same as they had during the tenth, respectively, averaging 10.2 and 7.5 entries; they seemed to remember little about the previous availability of water in the nook (Table 1). These findings were evaluated by subtracting the number of entries made by each rat during day 10 from the number it made during day 12; the *t* test for independent samples was then performed on the means of the difference scores, which were 2.62 and 15.20, respectively, for shocked and unshocked rats. The shocked group gave highly reliable evidence of poorer retention (*t* ratio = 5.13 at 16 degrees of freedom; $P < 0.001$).

The observations of primary importance to our study began just before the formal training trial of the eleventh day with a single 5 min recording of the baseline temperature

and then of the ECoG. Independent sets of flexible leads to a precision digital electronic thermometer (United Systems model 1502) and to a polygraph (Grass model 6-8-2) were attached sequentially to the calvarium-pedestal of each rat and then, after the thermal and the ECoG baselines had been recorded, were disconnected for attachment of the ECS leads. After the training trial and administration of ECS, alternate recordings of temperature and of the ECoG were made from time to time until the temperature of each rat was observed to increase to its baseline value. Cortical temperatures, which ranged from 36.4 to 38.0°C and averaged 37.2°C before ECS, decreased rapidly after the ECS treatment. The nadir of most of the shocked rats' temperatures occurred in about 15 min, ranged from 0.95° to 2.46°C below individual baselines, and persisted for about 30 min before a trend toward recovery was observed (Fig. 1). In all but one animal recovery to the baseline temperature occurred within 120 min. The one rat, however, exhibited a fluctuating hypothermia that persisted for nearly 10 h. Several days later, two of the eight experimental rats were selected randomly to provide additional baseline thermal data and were again subjected to the procedures of the eleventh day, except that they received sham ECS treatment. The brain temperatures of both animals during the 60 min recording session that followed were close to original baselines (Fig. 1).

When pre-ECS and post-ECS records of the ECoG and of the brain temperatures of individual animals were examined and compared, there was a marked covariation across time between thermal and electrocortical activity. The medium amplitude electrical activity of mixed, low-and-high frequencies that characterised the ECoG before ECS changed to the spiking and high-amplitude slow waves that characterise the electroencephalogram immediately after ECS⁸⁻¹⁰. A bout of isoelectric activity often followed the spiking and slow waves. Subsequently, until the cortical temperature began to increase, the ECoG was predominately one of slow wave, deltoid patterns. With initiation of thermal recovery there was a noticeable trend toward normalisation of the ECoG. Segments of the ECoG of a representative rat (Fig. 2) confirm to the eye the close correlation between electrical activity and temperature of the superficial cortex.

The major findings of our study are that: (i) a reversible cortical hypothermia is a consequence of an amnesia-inducing ECS; and (ii) the hypothermia is associated with an alteration of electrocortical activity. The degree of hypothermia

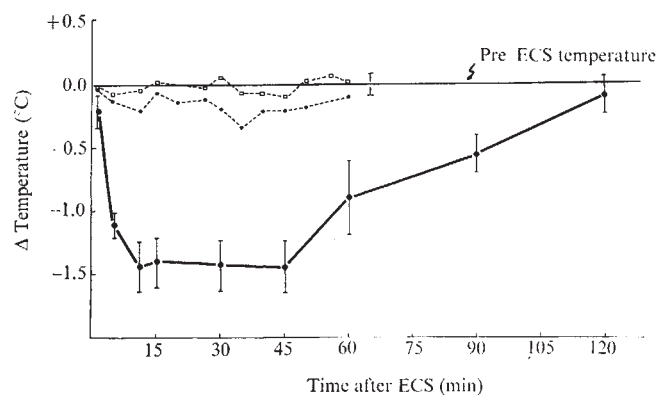


FIG. 1 The heavy line represents averaged decrements in degrees Celsius of cortical temperatures of eight rats after administration of a single electroconvulsive shock (ECS). The baseline of 0.0 °C is based on the averaged stabilised value of pre-ECS (basal) temperatures of the same animals. The dashed lines are individual thermal control data from two of the same rats as measured several days later after a sham-ECS treatment. The vertical lines extend one standard error above and below each mean.

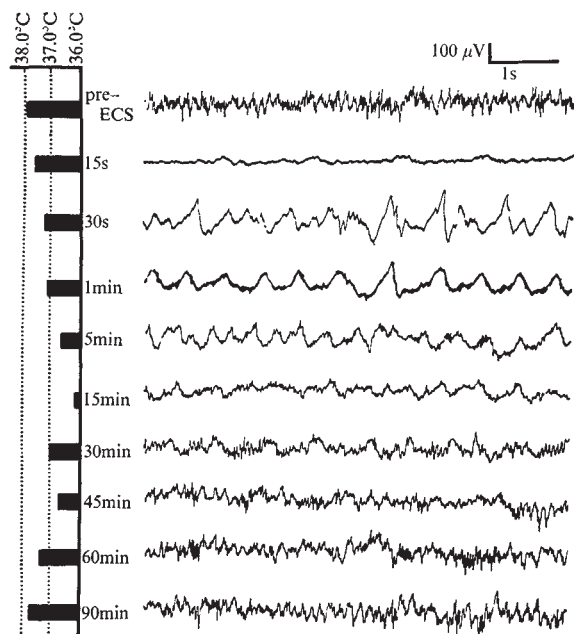


FIG. 2 Representative segments of the electrocorticogram (ECoG) of a rat are shown in relation to its cortical temperature as recorded within a few seconds of each other and as sampled across time before and after a single electroconvulsive shock. The ECoG was recorded from the superficial frontal cortex and was referred to an indifferent electrode near the midline of the cerebrum.

that was exhibited by our rats after ECS seems to be less severe than that which has been reported as necessary to produce amnesia by partial immersion of the murine animal in cold water¹⁻⁴. For example, Beitel and Porter reported² that a reduction of core temperature to 2° C was necessary to achieve reliable amnesic effects in mice. In contrast, Vardaris *et al.* found⁴ that a reduction of the rat's core temperature to 21.5° C produced not only retrograde amnesia but also frank seizure-like activity in the ECoG. In neither study, however, was cerebral temperature measured. Indeed, the absence of published information on simultaneously recorded thermal data of brain and core from animals that have been subjected to, and confirmed by behaviour, for amnesic treatments renders moot the question of a possible lack of parity in the degree of cortical hypothermia. That there is a highly reliable if moderate cortical hypothermia in the rat after ECS is both warrantably assertable and provocative of a more general question: do other amnesic agents such as the cortical¹¹ and hippocampal¹² spreading depressions also result in cerebral hypothermia? An affirmative answer would suggest that cerebral hypothermia is a causal antecedent and not merely a correlate in the stream of events whereby memory of recent occurrences is impaired by ECS and other amnesic agents. If cerebral hypothermia *per se* is critical to the annihilation of a newly forming engram, artificial maintenance of normal temperatures of experimental subjects immediately after ECS—achieved, for example, by immersing them partially in warm water or by carefully dosing them with microwave energy¹³—should preserve the engram. A corollary of the argument, that a mild hyperthermia may facilitate formation and maintenance of the engram, has already gained some support in the literature^{13,14}.

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ARVIN F. OKE

Laboratories of Experimental Neuropsychology,
Veterans Administration Hospital,
4801 Linwood Boulevard,
Kansas City, Missouri 64128

JOSEPH MENDELSON

Department of Psychology,
University of Kansas,
Lawrence, Kansas 66045

DON R. JUSTESEN

Department of Psychiatry,
University of Kansas Medical Center,
Kansas City, Kansas 66103

Received October 11, 1973; revised January 21, 1974.

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Short term memory and the EEG

STUDIES of human short term memory and physiological state have produced what seem to be paradoxical results, as efficient performance has been associated with low states of activation. This effect was first demonstrated by Kleinsmith and Kaplan¹ who measured electrodermal response (EDA) to paired associates. As yet, no similar relationship has been demonstrated for EEG activation and memory. Previous studies have been concerned merely with the gross differentiation of task activity and rest and simply show greater activation during learning^{2,3}. No-one has reported a detailed study of changes during verbal learning or of their relationship to subsequent recall.

The EEG was monitored during the acquisition and immediate recall of 9-digit strings presented in the auditory mode. After an initial 2 min rest period, 32 subjects each underwent 24 trials, for which instruction was minimal. The EEG was recorded, stored and averaged, using low frequency analysis in the manner described in earlier studies⁴ to yield an average abundance value per serial position. Because of the limitations of low frequency analysis, inter-digit interval within a series was 3 s. Each trial lasted 45 s with an EEG sample for each of the 15 × 3 s intervals (9 × 3 s per series, plus 3 s warning, plus 15 s for recall and inter-trial rest). The results reported here are largely concerned with 11.5–12.5 Hz; a complete report will be presented later for theta, beta and the remaining alpha frequencies.

Our key results were as follows. Task errors in recall increased monotonically as a function of serial position (ANOVA; $P < 0.001$) with an improvement in performance

for the final serial position ($P < 0.05$). Such an error curve is typical for tasks of this nature. At the same time, EEG abundance during acquisition diminished with serial position, such that high error in subsequent recall was associated with decreasing abundance (increasing arousal) in acquisition (ANOVA; $P < 0.01$). The monotonicity of the decrease in abundance (as assessed by Kendall's S) was positively correlated with number of recall errors in each of the serial positions, the strongest effects holding for the earlier positions (Spearman's correlations by ranks (ρ); $P < 0.05/0.01$). Similarly, total abundance during acquisition was positively related to subsequent recall (Pearson product-moment correlations; $P < 0.01$).

When subjects were divided into high and low recall error groups, the high recall error subjects showed greatest decreasing abundance in both the later stages of acquisition and at recall (ANOVA; $P < 0.05$). Both high and low error groups yielded recall curves which were identical in shape and parallel. But subjects with high recall errors even showed lower abundance (7.5–13.5 Hz) during a pre-test resting period and mean dominant frequency⁵ at that time correlated positively with subsequent error rate (Spearman ρ ; $P < 0.05$).

A within-subjects analysis showed that both high recall error trials and low recall error trials began with a monotonic decrease in EEG abundance (increasing arousal); but good performance (low error) trials then departed from this trend and were associated with higher abundance (t test; $P < 0.02$). The first eight trials were associated both with high recall errors in the early serial positions (ANOVA; $P < 0.01$) and decreased abundance when compared with the final 16 trials (ANOVA; $P < 0.05$). Finally, for all subjects, EEG abundance during between-trial rest was greater than during acquisition and recall ($P < 0.01$).

So far as we are aware, this is the first study to plot the systematic changes which occur in the human CNS during acquisition of material to-be-recalled, and to relate such changes to subsequent recall.

These results were wholly consistent for every type of analysis (subjects before the task, subjects during the task, serial position, high and low recall error trials, trials over time) since poor performance was associated with increased EEG arousal, even to the extent of enabling prediction of recall error rate before the task proper. The results were also compatible with those of Kleinsmith and Kaplan¹ who showed that in paired-associate learning, items which were associated with high EDA arousal were less well recalled in the short term. The finding that the EEG is more activated during the trials compared with the inter-trial resting periods, corroborates earlier work^{2,3}. Within the range of this increased activation, however, superior performance is associated with relatively lower activation levels.

Thus, short term memory tasks are an exception when compared with other tasks demanding sustained attention, such as vigilance tasks, where efficiency is positively related to level of activation. Short term memory tasks of the digit span type, typically demand brief periods of undivided attention, after which the subject may relax and prepare himself for the next trial. Such conditions of testing may well require lower levels of initial arousal to ensure efficiency. It should be noted that in the present task, ample time was available for rehearsal and for the self-monitoring of success or failure in retention.

A provisional interpretation of our results is that subjects who were already more highly aroused were less suited to good performance than subjects with lower initial arousal level. In addition, knowledge of failure in retention may have augmented the trend to increased arousal which in its turn may have reduced further the quality of performance.

The immediate memory span test is long-established within the repertoire of psychological measurement, with many

decades of use in intelligence testing. Further experimentation using the present paradigm may lead to an increase of our understanding of the neurological substrates of intellectual functioning.

ANTHONY GALE
DYLAN MARC JONES
ADRIAN SMALLBONE

*Department of Applied Psychology,
University of Wales Institute of Science and Technology,
Llwyn-y-Grant,
Cardiff CF3 7UX.*

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Visual attention affects brain blood flow

THE brain is extremely dependent on an uninterrupted supply of blood-borne nutrients because of its high metabolic rate. The effect of cerebral activity on the provision of blood to the brain, however, is not fully understood, particularly with respect to higher mental function. For human subjects, there is conflicting data concerning the possibility that intellectual effort increases the rate of flow of blood through the brain^{1,2}. We now report that cerebral blood flow may in part be regulated by the extent of mental activity. Our experimental animal was the chick, which has an anatomy such that minor changes in cerebral blood flow are readily detectable.

As the fibres of the optic tract are totally crossed over at the chiasm, each eye of the bird innervates only the contralateral optic lobe³. The cerebral hemispheres constitute the major associative areas of the chick and the innervation of each eye to these hemispheres, although indirect, is predominantly contralateral⁴. Furthermore, the absence of a major interhemispheric commissure, the corpus callosum, reduces interactions between the two symmetrical halves of the brain⁵. Consequently, the metabolic and vascular deficits caused by unilateral visual deprivation are largely confined to regions contralateral to the treated eye^{6,7}. Rapid and widespread changes in the rate of brain blood flow can be induced in chicks by occlusion of an eye by sewing the eyelids shut^{8,9}. However such studies cannot show whether reduced blood flow is due to reduced intensity of sensory input or to a decrease in behaviourally relevant information.

In our experiments two small cardboard blinkers were glued to the heads of 3-d-old chicks that had been fasted for the preceding day. The blinkers were positioned adjacent to each eye so that one eye could see only forward and the other only backward. Chicks were then placed in a smooth,

TABLE 1 Relative blood flow rates in the two sides of chick brain

	Ln F/B	
(a) 10 min pecking		
Optic lobes	+ 0.093 ± 0.021 ($P < 0.001$, $n = 31$)	
Cerebral hemispheres	+ 0.068 ± 0.021 ($P < 0.005$, $n = 31$)	
(b) 10 min non-pecking		
Optic lobes	+ 0.021 ± 0.023 (NS, $n = 23$)	
Cerebral hemispheres	+ 0.018 ± 0.026 (NS, $n = 23$)	

F, B, Blood flow through regions contralateral to forward and backward-looking eyes respectively (c.p.m. mg^{-1} tissue). Results are the mean of n birds $\pm$ s.e.; P , probability (Student's one-tailed t test); NS, non-significant.

TABLE 2 Difference in blood flow ratios of equivalent regions of pecking and non-pecking chicks

	(ln F/B) pecking—(ln F/B) non-pecking	
Optic lobes	+ 0.072 ± 0.031	(P < 0.025)
Cerebral hemispheres	+ 0.050 ± 0.034	(P = 0.08)

F, B, P are explained in Table 1. Figures are means of differences ± s.e.

white, circular bowl (25 cm diameter, 13 cm high) containing grains of chick feed. Under these conditions all birds immediately began to feed with straight, forward pecks, using the forward-looking eye to locate the grain. After 10 min of feeding, the relative rate of blood flow through the left and right optic lobes and cerebral hemispheres was determined by intracardiac injection of ^{125}I -iodoantipyrine, which is freely diffusible through both aqueous and lipid media¹⁰. After 10 s, the chick was decapitated and the brain rapidly dissected, weighed and placed into scintillation vials for assay of total radioactivity⁸. Data are expressed as c.p.m. mg⁻¹ tissue (wet weight).

Results were calculated as the ratio of the specific radioactivity in the brain region contralateral to the forward-looking eye (F) relative to the corresponding value for the paired region contralateral to the backward-looking eye (B). To avoid skewing data, the natural logarithm of this ratio was determined for each chick (ln F/B). This value was significantly above zero for paired optic lobes and also cerebral hemispheres (Table 1). Thus, the regions innervated by the forward-looking eye had a significantly greater rate of blood flow than regions associated with the backward-looking eye. This occurred even though the intensity of light exposure was very similar for each eye.

The experiment was repeated on a second group of chicks under identical conditions, except that the bowl was devoid of grain. In this situation, chicks do not peck or attempt to peck. The animals were occasionally prodded gently to encourage them to keep their eyes open. Two birds that did not keep their eyes open were not used. When birds had been in the bowl for 10 min, regional cerebral flow was again determined as in the grain-pecking group (Table 1). In this case there was no significant difference in blood flow through regions innervated by the forward-looking eye relative to the backward-looking eye.

A statistical comparison was made between data derived from birds pecking at grain relative to birds not receiving grain (Table 2). The asymmetry of regional blood flow in the pecking chicks was also significantly greater than corresponding values for non-pecking chicks. Thus the production of a difference in blood flow in the two sides of the brain was demonstrable only when the chicks were given grain to peck. Because pecking grain is not a learned behaviour, some other behavioural factor, such as attention or interest must be involved.

Such visual attention is sufficient to increase the rate of blood flow by 7–9% in the directly innervated optic lobes, and in the secondarily innervated cerebral hemispheres. These regions account for more than 75% of the total brain weight in the chick.

Many reports suggest that increased rates of macromolecular synthesis by brain are associated with learning or with exposure to an enriched environment^{11–13}. As attention is a significant feature of all learning and enriched-environment studies, the phenomena described here may underlie some of the metabolic events related to these processes. Such an association could be explained by variations in the supply of metabolites to the brain as the result of changes in brain blood flow. Such vascular mechanisms could also mediate the known relation between the quality of sensory input and cerebral development.

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STEPHEN C. BONDY
RALPH A. W. LEHMAN
JANET L. PURDY

Department of Neurology and
Department of Neurosurgery,
University of Colorado Medical Center,
4200 East Ninth Avenue,
Denver, Colorado 80220

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Bioconvection patterns in swimming microorganism cultures as an example of Rayleigh-Taylor instability

RATHER definite spatial patterns are observed in cultures of swimming microorganisms which are heavier than water and which tend to propel themselves toward the upper surface in response to some external stimulus; for example, gravity, light and chemicals. These patterns are characterised by falling fingers containing the organisms and are maintained by return upward swimming of the organisms. The patterns have been termed 'bioconvection' patterns¹ and have been known since 1848²; they are found in bacterial, flagellate^{3,4}, plankton⁵ and ciliate⁶ cultures.

Wager² studied the patterns formed in cultures of the flagellate *Euglena viridis*, among others, and was able to show that the tendency for these organisms to swim against gravity (negative geotaxis) was necessary for the pattern formation with live organisms. When such organisms are dead and form a layer at the bottom of the culture, a similar pattern of falling fingers can be obtained provided a sealed airless chamber of such a culture is very carefully inverted². In this case, of course, the pattern is not maintained.

Bénard (thermal) instability has been considered as a mechanism for this pattern and has been disproved¹. Indeed, the patterns are observed even when a stabilising temperature gradient is imposed. Another mechanism which has been considered and invalidated⁷ is the turnover effect, which is common in sucrose gradient preparations for centrifugation.

Quantitative observations have been made recently⁷ of the development of bioconvection patterns in cultures of *Tetrahymena pyriformis*, a ciliated microorganism which ex-

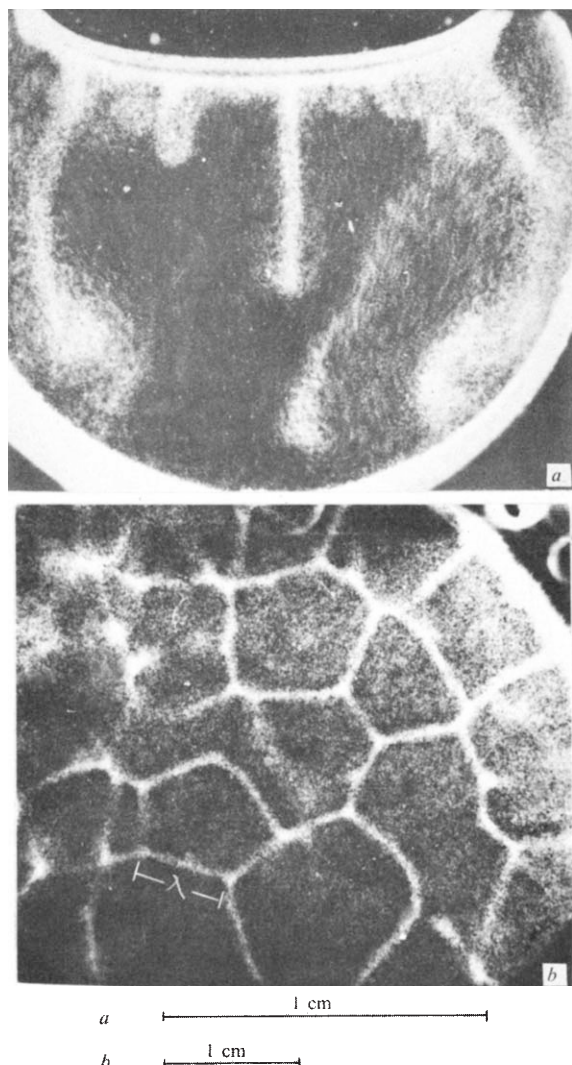


FIG. 1 *a*, A strobe-illuminated photograph of bioconvective sedimentation fingers descending from precipitation nodes; view is from the side. This *Tetrahymena* culture is enclosed in a 0.15 cm thick perfusion chamber of diameter 2.1 cm. The multiple flash mode utilised here gives one an impression of the bioconvective motion. *b*, Precipitation nodes and cross connections in a *Tetrahymena* culture as viewed from above. All light areas are masses of cells reflecting light. White lines are connections, and junctions are precipitation nodes.

hibits negative geotaxis. The system of falling fingers which characterises the pattern follows a period of accumulation in the upper layer of the culture. This layer typically develops nodes or bulges downward with fairly regular separations. The bulges then grow into falling fingers which descend with a velocity approximately twice the normal swimming velocity of the ciliate. Eventually flow connections develop between the nodes in the upper layer, particularly at the higher organism concentrations. The resulting familiar bioconvection pattern is shown in Fig. 1*a* which is a side view and Fig. 1*b* which is a view from above.

When the patterns are fully established the upper layer typically has a thickness of the order of 1.5 mm. It can be deduced readily from ref. 7 that the concentration of organisms in this upper layer is so large that the average distance between organisms is small compared with the layer thickness. As we shall see, the distances between the organisms is also small compared with other possible lengths involved in the physical model with which we are concerned here.

In this physical model the upper layer is a homogeneous

fluid with an excess density determined by the presence of organisms which are heavier than the medium. Recent measurements⁷ lead to values of the density increments for the upper layer over the lower layer and, typically, density increments are found to range from $\Delta\rho = 1.21 \times 10^{-4}$ to $\Delta\rho = 1.1 \times 10^{-3} \text{ g cm}^{-3}$.

The physical view of the process which we adopt here is as follows. As the microorganisms swarm beneath the upper surface, they form a fairly well defined layer of thickness h , which has a density ρ' which exceeds the density ρ of the underlying culture by a small amount $\Delta\rho$. This upper layer is taken to be a homogeneous fluid even though it contains microorganisms. As has been indicated, the approximation of homogeneity is justified for the situation in which the organisms are numerous and uniformly distributed so that their separations are very small compared with the thickness of the layer and with other lengths involved in the physical model such as λ (the internodal distance defined below).

The physical situation is gravitationally unstable, of the sort which is familiar in fluid mechanics as Rayleigh-Taylor instability⁸. The unusual feature of the present conditions is that the gravity force is so small that viscosity becomes quite important. As is usual in a stability problem, the initial growth of a small disturbance is analysed, and for the present unstable case an initial disturbance grows like e^{nt} , where t is the time and n is a function of the wavelength of the disturbance. The exact treatment of this two-fluid problem, where the upper fluid has a small thickness, h and the lower fluid a large thickness has apparently not yet been published, but it is a relatively straightforward matter which, however, has considerable algebraic com-

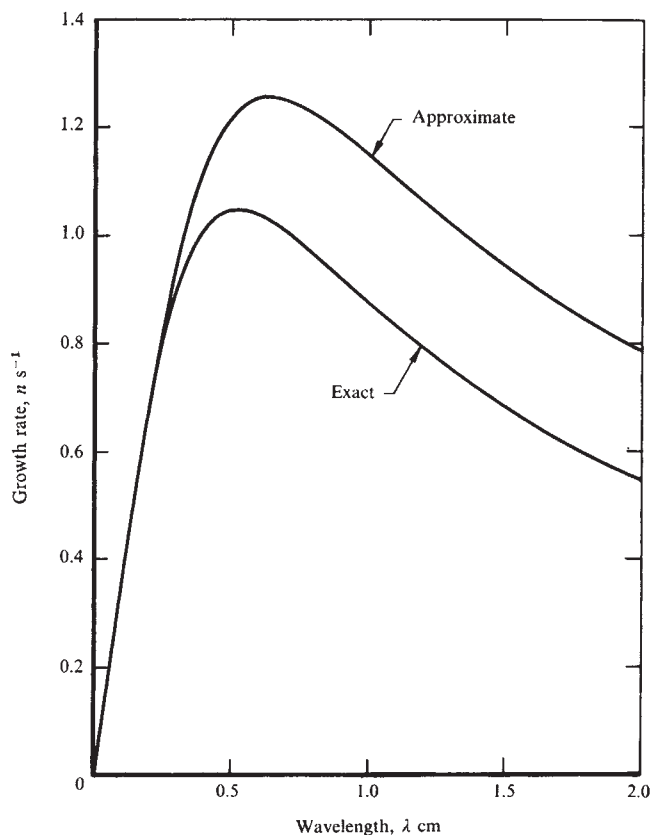


FIG. 2 The ordinate gives n , the growth parameter of the initial interface wave disturbance which has amplitude increasing as e^{nt} . The maximum n is at $\lambda_m = \lambda_{\text{theor}}$ which occurs here at 0.65 and 0.525 cm. These theoretical curves are obtained for $\Delta\rho = 9.0 \times 10^{-4} \text{ gm cm}^{-3}$ with an upper layer depth of $h = 0.13 \text{ cm}$. The λ_m condition corresponding to this model is illustrated in Fig. 3.

TABLE 1 Measured and theoretical values for two *Tetrahymena* cultures

C_0 (cells cm^{-3})	3×10^4	2.71×10^5
h (cm)	0.15	0.13
C_l (cells cm^{-3})	0	5.6×10^5
C_u (cells cm^{-3})	7.1×10^4	1.4×10^6
$\Delta\rho$ (g cm^{-3})	1.21×10^{-4}	1.08×10^{-3}
λ_{meas} (cm)	~ 1.0	0.655
λ_{theor} (cm)(exact theory)	0.80	0.525
λ_{theor} (cm)(approximate theory)	1.1	0.65

The range of parameters shown is characteristic of *T. pyriformis* culture systems and although stated values of C_0 are exceeded in both directions no nodes appear at values below the smaller figure and cultures at values above the larger figure were not analysed. The symbols are defined in the text.

plications. Although the exact solution of this problem has been achieved⁹, the results are available in numerical form only. The results of the exact calculation for a layer thickness $h = 0.13$ cm and a density excess in the upper layer $\Delta\rho = 9.0 \times 10^{-4}$ g cm^{-3} is shown in Fig. 2. It can be shown⁹ that a simple approximation for the amplitude behaviour of a disturbance $a_k(t) = a_k(0)e^{nt}$ is given by

$$n^2 + 2\nu k^2 n - \sigma^2 = 0 \quad (1)$$

where

$$\sigma^2 = \frac{\rho' - \rho}{\rho' + \rho \coth kh} gk \quad (2)$$

Here $k = 2\pi/\lambda$ is the wave number, g is the acceleration of gravity and λ is the internodal wavelength. Figure 2 also shows n as a function of λ as determined from the approximate theory. Both the exact and the approximate theory show that n has a maximum at about the same value of λ . It is evident that there is a relatively small band of wavelengths about this maximum which leads in the growth of the fingers. The λ for which n is maximum, that is λ_m , should give the most frequently observed separation distance between the fingers. While the theory is limited to small amplitudes, it is clear that the wavelengths which lead initially will continue to lead.

For comparison with these theoretical ideas *T. pyriformis* was grown axenically and cultures were poured into dishes 4×5 cm in cross section. Fluid depth was maintained at 1.2 cm. Culture samples from the upper and lower layer were extracted by a device similar to that described previously⁷. Sample concentrations were determined with a Sedgwick-Rafter counting chamber and culture concentrations with a Coulter counter. Swarm layer thickness, h , was determined from photographs. The density increment was found from

$$\Delta\rho = (C_u - C_l)(m_T - V_T\rho_M),$$

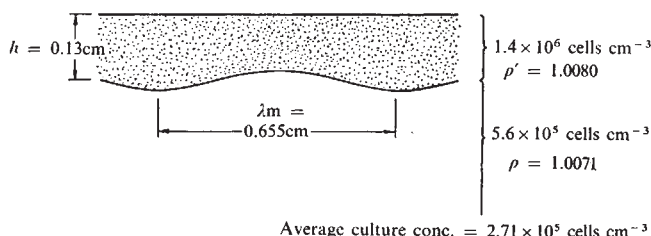


Fig. 3 Rayleigh-Taylor instability parameters measured for one culture of *T. pyriformis*. The $\lambda_m = 0.655$ is an average nearest internodal distance obtained from nodes at least 1 cm from the chamber wall.

where C_u and C_l are upper layer and lower layer medium cell concentrations respectively, m_T and V_T are the mass and volume respectively of *T. pyriformis*, and ρ_M is the culture fluid density. Parameters for a sample culture are presented in Fig. 3.

Table 1 summarises two sets of measurements near the extremes of the observed culture concentration range. The table also gives a comparison of the observed internodal distance with the values predicted by the present theory.

The agreement between the theoretical and observed maximum values is good. We conclude that Rayleigh-Taylor instability is the mechanism for the observed bioconvection in *T. pyriformis* cultures and may also explain sedimentation patterns in other microorganism swarms insofar as they are not disturbed by surface wind or by thermal gradients.

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MILTON S. PLESSET
HOWARD WINET

California Institute of Technology,
Pasadena, California 91109

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Decrease of polysomes in *Tetrahymena* after synchronisation shocks

THE rate of protein synthesis in eukaryotic cells can change rapidly as a result of translational control such as the inhibition of protein synthesis during mitosis¹ or starvation²⁻⁴. Under these conditions a reversible inhibition of the initiation step of protein synthesis decreases the rate at which ribosomes become attached to messenger RNA. The continuing runoff of ribosomes from messenger during protein chain elongation and termination depletes the percentage of the total ribosomes which are in the form of polysomes and are synthesising protein.

Tetrahymena is a convenient cell in which translational control can be studied by measuring the percentage of polysomes under a variety of conditions. Of particular interest are the effects of temperature shifts which synchronise cell division in *Tetrahymena* cultures^{5,6} and which cause rapid and reversible inhibition of the initiation step in mammalian cells⁷⁻¹⁰. This report shows that temperature shifts, hypoxia and deprivation of nutrients all cause rapid, reversible decreases in the percentage of polysomes in *Tetrahymena* due to an inhibition of initiation. In previous reports decreases in the percentage of polysomes in this organism were noted only after long periods of cold shock¹¹ or starvation¹², and fractionation techniques were used which permitted considerable degradation of the polysomes.

Tetrahymena pyriformis (strain T) growing at 28° C in 2% proteose peptone-0.2% yeast extract was harvested in exponential phase and lysed with detergent in the presence of 100 μg heparin ml^{-1} . Centrifugation of the lysates on sucrose density gradients showed that about 75% of the

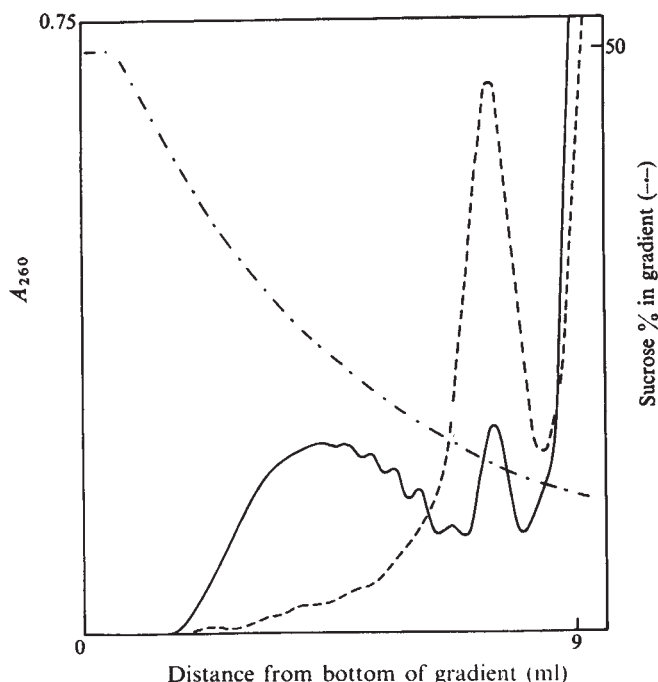


Fig. 1 Sucrose gradient centrifugation of *Tetrahymena* polysomes. Cells were collected by chilling the cultures ($0.5 - 1 \times 10^6$ cells ml^{-1}) and centrifuging at 0°C , washed once with 1 mM MgCl_2 — 10 mM KCl — 10 mM Tris-HCl (pH 7.4) — 40 mM NaCl, suspended in 5 mM MgCl_2 — 5 mM mercaptoethanol — 20 mM Tris-HCl (pH 7.4) — 100 mM KCl — 250 mM sucrose containing 100 μg heparin ml^{-1} , and lysed by adding Nonidet-P40 to 0.2% and sodium deoxycholate to 1.0%. The lysate was centrifuged (12,000g, 15 min) and portions of the supernatant (equivalent to approximately 3×10^5 cells) were layered over non-isokinetic sucrose gradients¹³ (8.5 ml 10% to 50% sucrose over 0.5 ml 50% sucrose) containing 5 mM MgCl_2 — 10 mM Tris-HCl (pH 7.4) — 100 mM KCl . After centrifugation at 4°C (75 min, 30,000 r.p.m.) the distribution of ribosomes and polysomes was determined by pumping the gradient from the bottom of the tube through an LKB 4 mm square section flow cell. The extinction at 260 nm was continuously recorded with a Gilford model 2000 recording spectrophotometer. Treatment with pancreatic ribonuclease (25 μg ml^{-1} , 10 min at 25°) was carried out just before layering on the gradient. —, control; ---, RNase.

ribosomes were in the form of polysomes which sedimented more rapidly than the free (80S) ribosomes, but which were degraded to 80S structures by added pancreatic ribonuclease (Fig. 1).

The effects of inhibitors of translation demonstrated that the polysomes in the cell were steady-state structures resulting from a balance between the rate of ribosome attachment (initiation) and the rate of ribosome runoff. Cells incubated in growth medium containing ^{14}C -leucine (0.5 μCi ml^{-1} , 50 μCi μmol^{-1}) incorporated radioactivity into hot trichloroacetic acid-precipitable material at a linear rate for 60 min. This incorporation was inhibited 70% by 50 μM 2-(4-methyl-2,6-dinitroanilino)-N-methyl propionamide (MDMP), an inhibitor of initiation^{14,15}, and 50% by cycloheximide (0.5 μg ml^{-1}) which inhibits chain elongation. Figure 2 shows that 50 μM MDMP decreased the proportion of polysomes to 30% in 10 min. This effect was reversed by low concentrations of cycloheximide which, by partially inhibiting chain elongation, slow down the transit of ribosomes over the messenger, so preventing the depletion of the polysomes by the initiation inhibitor (see for example ref. 1). In the same way cycloheximide added to control cells increased the percentage of polysomes to 85–90%.

In contrast with these rapid effects other experiments (results not shown) demonstrated that 50 μg actinomycin ml^{-1} (which inhibited ^{14}C -uracil incorporation into the RNA

of intact cells by 95%) affected the percentage of polysomes much more gradually. Thus the percentage of polysomes fell from 76% to 70% after 30 min, and to 40% after 90 min, while ^{14}C -leucine incorporation was approximately 50% inhibited after 90 min. This suggests that rapid changes of the percentage of polysomes are unlikely to depend on changes in the rate of RNA synthesis.

When cells in growth medium were subjected to heat shock by raising the temperature to 34°C for 10 min the proportion of polysomes decreased to 45% (Fig. 3a and Table 1) and ^{14}C -leucine incorporation by the intact cells was 33% inhibited. When the cells were returned to 28°C , recovery was essentially complete and was unaffected by the presence of actinomycin (50 μg ml^{-1}). The decreased proportion of polysomes at 34°C reflected an altered balance between attachment and runoff of ribosomes, and cycloheximide (0.5 μg ml^{-1}) restored the percentage of polysomes to approximately the normal level presumably by slowing down the runoff of ribosomes (Table 1). A decrease in the number of polysomes could result from a decreased rate of ribosome attachment to mRNA (initiation) or from an increased transit rate of ribosomes over mRNA. Since protein synthesis was inhibited at 34°C the decreased percentage of polysomes probably reflected an inhibition of initiation. After 30 min at 34°C recovery at 28°C was incomplete (Table 1) which suggests a loss of messenger during the longer period at 34°C . It has been shown that at this temperature RNA in *Tetrahymena* breaks down more rapidly¹⁶ and stability of messenger (measured subsequently at 28°C as the capacity for protein synthesis in the presence of actinomycin) is decreased¹⁷.

Shifting the cells to 10°C inhibited protein synthesis by 80% and affected the polysomes in the same way as heat shock (Table 1 and Fig. 3b). Thus the percentage of polysomes was approximately 40% after 15 min or 60 min

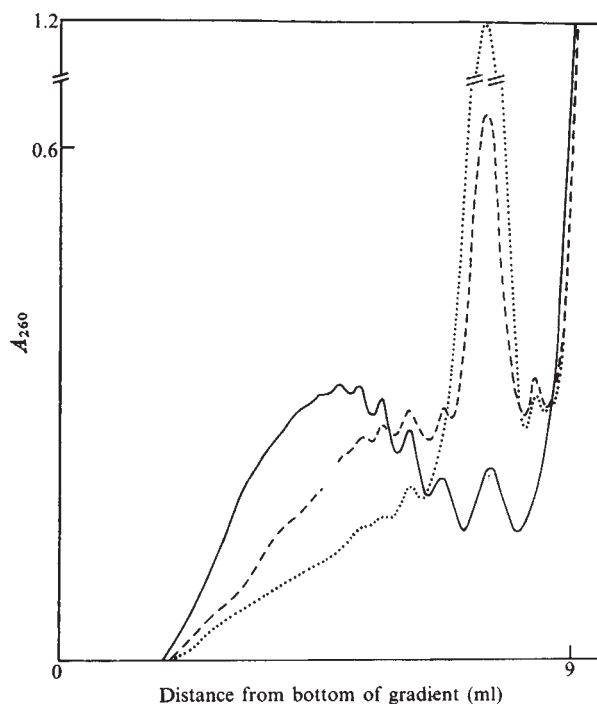


Fig. 2 Effect of MDMP (---), cycloheximide (—) and MDMP plus cycloheximide (····) on *Tetrahymena* (polysomes). Cell cultures in growth medium were incubated at 28°C for 10 min with added cycloheximide (0.5 μg ml^{-1}) or MDMP (50 μM). Samples of cell lysate (equivalent to approximately 5×10^5 cells) were centrifuged as in Fig. 1. Values for percentages of polysomes determined from the absorbancy tracing at 260 nm are cycloheximide 85%, MDMP 30%, MDMP + cycloheximide 61%. The values for controls without inhibitor were 72–76%.

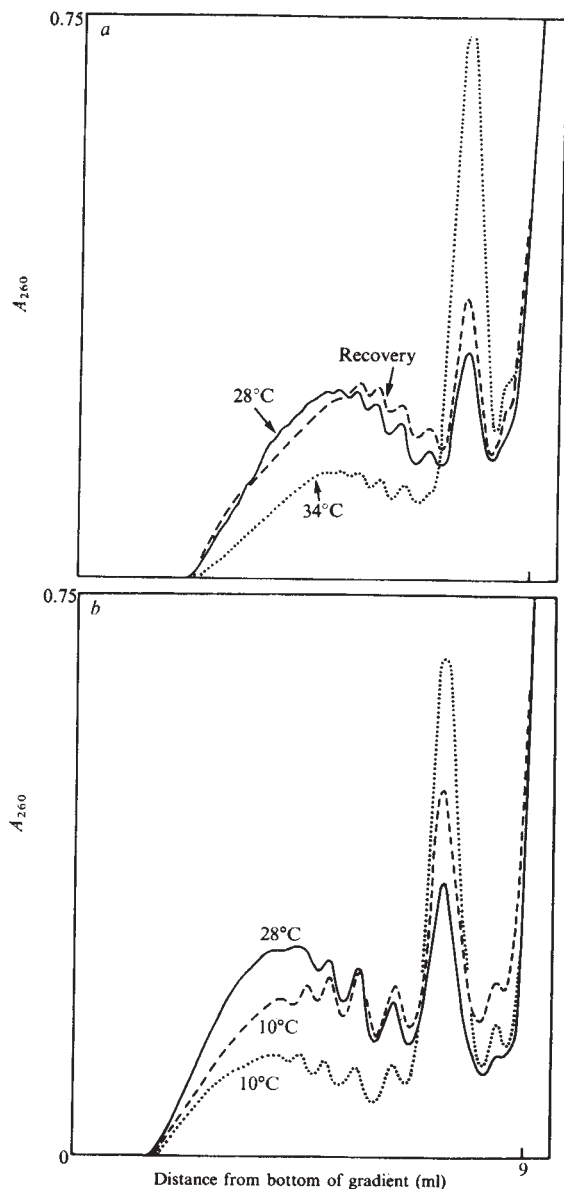


FIG. 3 Effect of temperature shifts on *Tetrahymena* polysomes. Cultures in growth medium were incubated as follows. *a*, (Heat shock): at 28°C, 10 min at 34°C, or 10 min at 34°C followed by recovery for 10 min at 28°C. *b*, (Cold shock): at 28°C, 15 min at 10°C, or 15 min at 10°C in the presence of cycloheximide ($0.5 \mu\text{g ml}^{-1}$) (— — —).

of cold shock and this decrease was prevented by cycloheximide. Recovery at 28°C was rapid and virtually complete even after 60 min at 10°C. This is in agreement with the finding that messenger is stable at low temperatures¹⁷.

Hypoxia has been used to synchronise *Tetrahymena*¹⁸ and may have an effect analogous to starvation of energy substrates⁴ which, like amino acid starvation^{3,4}, inhibits initiation of protein synthesis in mammalian cells. After 30 min of hypoxia in growth medium or 10 min of starvation in a saline medium the polysomes were decreased to about 40% (Table 1). In both cases cycloheximide prevented the depletion of polysomes and recovery was rapid though incomplete when normal conditions were restored. If the unlikely possibility that hypoxia or starvation increased the rate of ribosome transit on mRNA is excluded, then the decrease in the number of polysomes under these conditions must have resulted from a reversible inhibition of the initiation step.

Therefore, as in mammalian cells, temperature shifts and starvation seem to decrease the percentage of polysomes in

TABLE 1 Percentage of Polysomes in *Tetrahymena* under Various Conditions

Incubation conditions	% of polysomes		
	Incubation without cycloheximide	Incubation with cycloheximide	Incubation without cycloheximide, then 10 min at 28°C in growth medium
34°C for 10 min	45	78	74*
34°C for 30 min	46	—	62
10°C for 15 min	41	71	—
10°C for 60 min	40	—	71*
Hypoxia, 30 min	42	73	64
Starved, 10 min	42	60	64†

The percentage of polysomes was determined as in Fig. 2 after temperature shift (Fig. 3), hypoxia (cultures in growth medium at 28°C gassed with nitrogen) or starvation (cells suspended in 1 mM MgSO_4 — 5 mM potassium phosphate (pH 7.0) — 45 mM NaCl at 28°C). These conditions were modified as shown by including cycloheximide ($0.5 \mu\text{g ml}^{-1}$) or by permitting recovery by subsequent incubation for 10 min in growth medium at 28°C. Values are averages of two or more results (which agreed within approximately 5%). In control cells (growth medium at 28°C) the proportion of polysomes was 73–77%.

* Results after recovery in the presence of actinomycin ($50 \mu\text{g ml}^{-1}$) were essentially the same.

† Similar results were obtained when an equal volume of double-strength growth medium was added or when the cells were centrifuged at 0°C and resuspended in growth medium at 28°C.

Tetrahymena by inhibiting initiation. Messenger not engaged in polysome formation may as in *Escherichia coli*^{19,20} be more susceptible to degradation, especially at higher temperatures. In this way inhibition of initiation during relatively short periods at 34°C or longer periods at 10°C could contribute to that depletion of messenger for proteins related to cell division which is the probable basis of synchronisation⁵.

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H. G. KLEMPERER
V. A. ROSE

Department of Biochemistry,
University of Birmingham,
Birmingham B15 2TT

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New genetic and physicochemical data on structure of dinoflagellate chromosomes

THE majority of free-living dinoflagellates differ from other eukaryotes in possessing high chromosome numbers¹, large amounts of DNA² per cell, uniform chromosome size and in their apparent lack of histone proteins³. One of their most interesting properties which they share along with the euglenids⁴ is the condensed state of their chromosomes throughout the cell cycle⁵.

These chromosome properties immediately suggest three simple models for nuclear DNA organisation: (1) a normal pattern of alternation of haploid and diploid generations with each haploid chromosome non-identical; (2) polyploidy, having three or more sets of homologous chromosomes and (3) polyteny, a multistranded condition of chromosomes found for example in *Drosophila* salivary glands. Haapala and Soyer⁶ have recently proposed a diploid-polyteny model for the organisation of the dinoflagellate nucleus. On the basis of our work on *Cryptocodinium cohnii* (Seligo) Chatton in Grassé, 1952 (GC strain, Indiana University Culture Collection No. 1649), and other dinoflagellates we take issue with this model for the following reasons. Haapala and Soyer⁶ assumed dinoflagellates to be diploid. To date all except possibly *Noctiluca*⁷ have been cytologically determined to be haploid⁸⁻¹². Our chemical mutagenesis studies using N-methyl-N'-nitro-N-nitrosoguanidine have demonstrated that nutritional and carotenoid deficient mutants can be obtained at a frequency similar to bacteria¹³. (Chemical mutagenesis of *C. cohnii* was carried out by the method of Adelberg *et al.*¹³, with the following modifications: temperature = 25° C, final concentration of nitrosoguanidine = 20 µg ml⁻¹, carried out in M buffer (342 mM NaCl, 28 mM MgSO₄, 2 mM CaCl₂, 9 mM KCl, 8 mM 4-morpholine-ethane sulphonic acid, final pH = 6.0). Treatment time = 6 min. Under these conditions, the mutation frequency for purine and pyrimidine requiring strains was in the range of 0.17-0.83% of survivors and for carotenoid deficient strains in the range of 0.2-0.29% of survivors. Adelberg *et al.*¹³ obtained valine resistance in *E. coli* K12 at a maximum frequency of 0.20% survivors.) Only a haploid nuclear condition would be consistent with the frequency of mutations obtained. To find a viable mutant of diploid organism one would have to mutate the same gene on both homologous chromosomes without causing additional lethal mutations elsewhere in the genome. So, the frequency of obtaining conditionally lethal mutants is very small in a diploid organism. For the above reasons mutational data also rule out simple polyteny and polyploidy, where every single gene on every chromosome is polytenised or where every chromosome is present in two or more copies. Our DNA renaturation kinetic studies on *C. cohnii* also rule out polyteny and extreme polyploidy, where every chromosome is exactly the same. From DNA measurements on exponentially growing cells we find 6.9 µg per 10⁶ cells by two spectrofluorometric methods: ethidium bromide¹⁴, and diaminobenzoic acid^{5,15}, which agree with the reported value of 6.9 µg per 10⁶ cells obtained with the diphenylamine method¹⁷. Using the acetocarmine technique¹⁸ we have obtained counts of 107 and

110 chromosomes for *C. cohnii* in close agreement to the reported¹⁹ value of 99. Assuming all of the chromosomes have roughly the same amount of DNA, we calculate 4.2×10^{10} daltons per chromosome. If extreme polyploidy were the case, then the slowest renaturing component of *C. cohnii* DNA would renature at a rate no more than twenty times slower than *Escherichia coli* strain C600 DNA which has about 2.2×10^9 daltons per chromosome¹⁴. Figure 1 presents renaturation kinetic measurements of *C. cohnii* and *E. coli* DNA. Although it is obvious that there is a component comprising roughly 60% of the dinoflagellate DNA which renatures at a rate comparable to *E. coli* the remaining 40% is not renatured at C_0t value sixty times greater than the $C_0t_{1/2}$ of

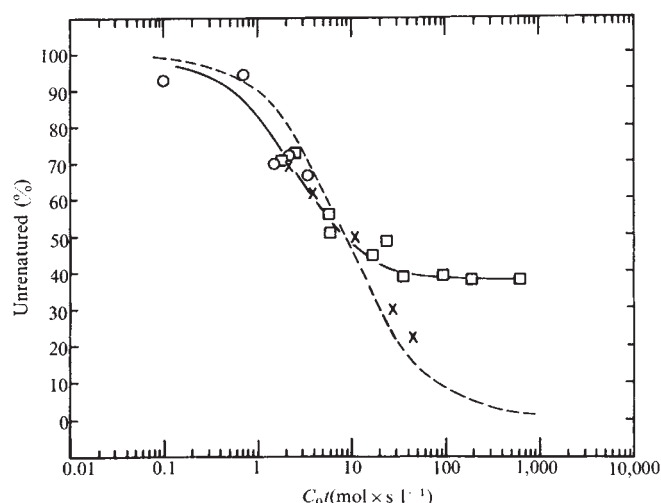


FIG. 1 The kinetics of re-association of the DNA from the dinoflagellate *C. cohnii* compared with that of the bacterium *E. coli* C600. The DNA of both organisms was isolated by the method of Marmur²⁰ and tested for purity by CsCl equilibrium density ultracentrifugation and optical melting analysis. DNA was sheared by sonication to fragments of 500 ± 50 nucleotide pairs (as measured by sedimentation rate in the Spinco Model E ultracentrifuge). Samples of DNA in 120 mM phosphate buffer were denatured by heating to 100° C for 5 min and then allowed to renature for various times at $60 \pm 1^\circ$ C. The samples were then passed over a hydroxyapatite column at 64° C to separate quantitatively single and double stranded DNA. DNA concentrations were: $\times$, 190 µg ml⁻¹ *E. coli* DNA; $\square$, 500 µg ml⁻¹ *C. cohnii* DNA; $\circ$, 100 µg ml⁻¹ *C. cohnii* DNA.

E. coli DNA. If *C. cohnii* were 500 times polytene as proposed by Haapala and Soyer⁶, the slowest component of the DNA should renature no more than four times more slowly than *E. coli* DNA; that is, $C_0t_{1/2} \leq 40$. Our measurements indicate the $C_0t_{1/2}$ of the 60% component of the dinoflagellate is about 1, and its kinetic complexity is estimated to be 2.5×10^8 daltons ($1/10 \times$ kinetic complexity of *E. coli* DNA). The amount of DNA comprising this component is 2.4×10^{12} daltons per nucleus, thus the sequences are repeated about 10,000 times. Repeated sequences of such high complexity are unusual, and might be important in establishing the evolutionary history of dinoflagellates. The remaining 40% of the DNA is not appreciably renatured at C_0t 600. Since the C_0t plot representation of the renaturation of the 40% component must have a finite slope no steeper than -20 (% unrenatured/log C_0t) as predicted from theory, the $C_0t_{1/2}$ of this slowly-renaturing component can be no less than 3000; and thus the DNA of this component can be repeated no more than ten times per cell. Thus our renaturation kinetics and chemical mutagenesis studies do not support the conclusions of Haapala and Soyer⁶ of a diploid-polytene nature of the dinoflagellate nucleus. Their suggestion of multiple circular DNA molecules composing a dinoflagellate chromosome is

however, very intriguing. Whether each chromosome contains one or several pieces of DNA may be tested using the viscoelastic technique^{14,21}.

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T. M. ROBERTS
R. C. TUTTLE
J. R. ALLEN
A. R. LOEBLICH, III
L. C. KLOTZ

Department of Biochemistry and
Molecular Biology and
Department of Biology,
Harvard University,
Cambridge, Massachusetts 02138

Received September 4, 1973.

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divided by the shell volume to give a 'shell density.' 'Mean densities' for spheres of radii 2 Å, 4 Å, 6 Å, and so on, were also calculated by dividing the total mass enclosed within each sphere by the sphere volume.

Results for five proteins are shown in Figs 1 and 2. The innermost shell densities vary widely due to their few atoms. Farther out in the molecule shell and mean densities of 1.02–1.27 and 1.14–1.22 g cm⁻³, respectively, are found. When shells become large enough to include solvent molecules (which were not included in the calculations), the shell density declines. The outer radius of the shell beyond which this decline begins is called R_{max} ; all points within R_{max} are presumed to lie entirely within the molecular cavity. The

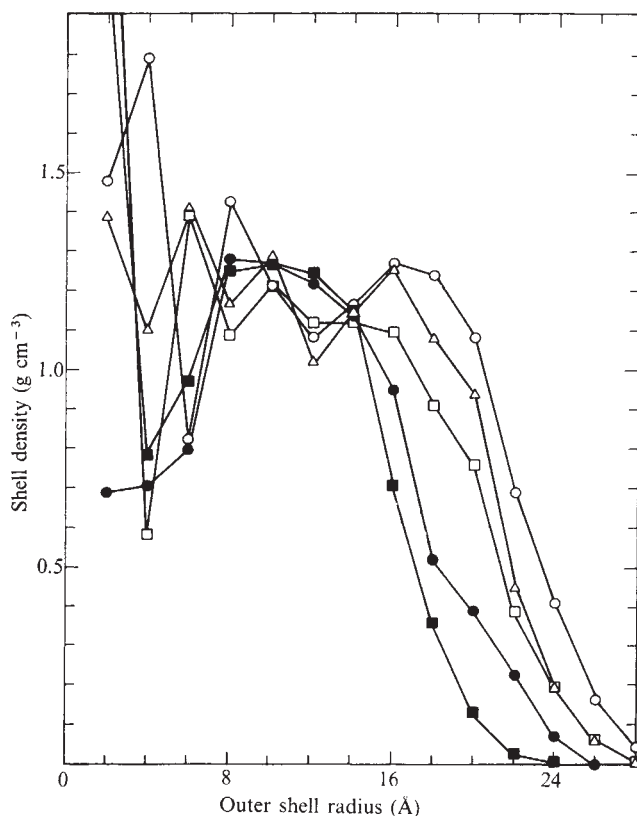


Fig. 1 Dependence of shell densities on shell radius. O, Carboxypeptidase; Δ , subtilisin; $\square$, chymotrypsin; $\bullet$, myoglobin; $\blacksquare$, lysozyme.

Protein densities from X-ray crystallographic coordinates

DENSITIES of the interiors of nine protein molecules were calculated using atomic coordinates obtained by X-ray crystallography. The resulting values, obtained for spherical regions of the molecules surrounding their centres of mass, are 10–25% below the apparent densities of the same molecules in solution. These regions include 20–50% of the total molecular mass. The densities calculated were 1.09–1.25 g cm⁻³ whereas the observed apparent solution densities of these proteins are 1.33–1.42 g cm⁻³.

Calculations were performed as follows. All atoms in each molecule were identified and assigned appropriate masses, including hydrogens, which are not generally located by protein X-ray crystallography (thus, all α -carbons except proline were given mass 13; all peptide nitrogens except proline, 15; the alanine β -carbon, 15; the lysine epsilon amino nitrogen, 17, and so on). The molecular centre of mass was located and its distance from each atom was determined. The masses of all atoms within shells 0–2 Å, 2–4 Å, 4–6 Å, and so on, from the centre of mass were added, and the sum

mean density of the sphere of radius R_{max} , calculated as just described, is denoted by ρ_{calc} , and the observed apparent density of the protein, as obtained from solution density measurements, is ρ_{obs} . (In two instances ρ_{obs} will be the value calculated by the Cohn-Edsall method, which generally agrees well with solution density values¹.)

Table 1 gives R_{max} , ρ_{calc} and ρ_{obs} for nine proteins. Column 6 shows that ρ_{calc} is 0.10–0.36 g cm⁻³ below the corresponding ρ_{obs} (the mean difference is 0.21). Column 3 gives the fraction of the total molecular mass within the sphere of radius R_{max} . The abnormally low densities refer to only the central 20–50% of the molecule.

Three explanations for this discrepancy are conceivable. (The possibility that the apparent protein density in the crystal differs to this extent from the density in solution seems to be excluded by the measurements of Low and Richards^{2,3} and of McMeekin, Groves and Hipp⁴.)

(a) The protein has the density ρ_{calc} throughout, but may be surrounded by an abnormally dense water jacket. To estimate the density in this jacket an equivalent radius, R_{eq} , was calculated for two of the proteins from the known

TABLE 1 Density calculations for nine proteins

(1) Protein	(2) $R_{\max}$ (Å)	(3) Mass fract.	(4) ρ_{calc} g cm ⁻³	(5) ρ_{obs} g cm ⁻³	(6) $\rho_{\text{obs}} - \rho_{\text{calc}}$	(7) n_+ (ref. 7)	(8) n_- (ref. 7)	(9) $\Delta\rho_{\text{elect}}$ g cm ⁻³	(10) ρ_{outer} g cm ⁻³	(11) $\rho_{\text{outer}} - \rho_{\text{calc}}$	(12) $n_{\text{H}_2\text{O}}$
Myoglobin*	12	0.291	1.19	1.35 ⁸	0.16	23	22	0.046	1.43	0.24	117
Lamprey haemoglobin*	14	0.477	1.15	1.34 ⁸	0.19	18	24	0.042	1.56	0.41	128
Carboxypeptidase	18	0.527	1.23	1.33 ^{9†} 1.39 ³	0.10 0.16	26	26	0.029	1.47 1.63	0.24 0.40	148 240
Chymotrypsin	14	0.320	1.14	1.37 ¹⁰	0.23	17	14	0.024	1.51	0.37	228
Chymotrypsinogen	14	0.294	1.09	1.38 ⁸	0.29	18	14	0.024	1.56	0.47	304
Lysozyme	12	0.381	1.25	1.42 ¹¹	0.17	17	10	0.038	1.55	0.30	96
Ribonuclease	10	0.194	1.05	1.41 ^{12‡}	0.36	14	10	0.035	1.54	0.49	195
Staphylococcal nuclease	12	0.325	1.20	1.39 ^{13§}	0.19	28	19	0.056	1.51	0.31	122
Subtilisin	16	0.447	1.18	1.38 ^{14§}	0.19	23	25	0.033	1.58	0.39	209

* These include contributions of haem groups.

† An assumed value, included to show, by comparison with the alternate value, the sensitivity of columns (10)–(12) to the value of ρ_{obs} .

‡ Same value is obtained when ρ is calculated from the amino acid composition by the Cohn-Edsall method¹.

§ Value calculated from amino acid composition by the Cohn-Edsall method¹.

TABLE 2 Density of water jacket required to raise the calculated densities, ρ_{calc} , to the observed apparent solution densities, ρ_{obs}

Jacket thickness (Å)	3.0	6.0	9.0	15.0
ρ_{jacket} { Myoglobin g cm ⁻³ Carboxypeptidase	1.624 1.547	1.467 1.426	1.418 1.387	1.381 1.357

total molecular mass and ρ_{calc} . ($R_{\text{eq}} = (3M/4\pi N_0 \rho_{\text{calc}})^{1/3} = 18.08$ Å for myoglobin, with $M = 17756$, $\rho_{\text{calc}} = 1.19$; $R_{\text{eq}} = 22.28$ Å for carboxypeptidase, with $M = 34337$, $\rho_{\text{calc}} = 1.23$.) The required jacket density is then $\rho_{\text{jacket}} = [\rho_{\text{obs}}(R_{\text{eq}} + t)^3 - \rho_{\text{calc}}R_{\text{eq}}^3]/[(R_{\text{eq}} + t)^3 - R_{\text{eq}}^3]$, t being the jacket thickness. The value $t = 3.0$ Å is equivalent to a jacket about one water molecule thick. Table 2 shows that water densities of 1.35–1.60 are required to bring ρ_{calc} to the observed value if the jacket is 1–5 water molecules thick. (ρ_{obs} was given values 1.35 for myoglobin and 1.33 for carboxypeptidase.)

Electrostriction by charged groups could account for only a portion of such a density increase. Columns 7, 8 and 9 of Table 1 give the numbers of positively and negatively charged side chains on each protein molecule and the apparent density increase, $\Delta\rho_{\text{elect}}$, resulting from a 10 cm³ mol⁻¹ electrostrictive contraction per charged group. Evidently $\Delta\rho_{\text{elect}}$ is 0.025–0.05 whereas the discrepancy, $\rho_{\text{obs}} - \rho_{\text{calc}}$, is 3.4–12 times greater than this.

A structureless water jacket with $\rho = 1.3$ –1.6 might be detectable as a halo of high electron density on X-ray Fourier maps; we understand that this is not observed.

(b) The outer portion of the molecule may have a higher density than the central region. This difference would be expected if the interior of the molecule contains mostly hydrophobic groups and if polar groups tend to be found near the molecular surface. (Solid straight chain paraffins and benzene have densities of about 1.0. Most proteins, however, have peptide groups buried in their interiors as β -sheets and α -helices. Cohn and Edsall give 20 cm³ as

the mole volume of the –CO–NH–group, equivalent to 2.15 g cm⁻³. Thus the interior density should be somewhat above 1.0.) Apparent densities, ρ_{outer} , of the outer regions were calculated by dividing the difference between the total molecular weight and the mass enclosed within R_{max} by the difference between the volume bounded by R_{max} and the total molecular volume, as calculated from the molecular weight and the observed partial specific volume in solution. These are shown in Table 1, column 10. Values range from 1.43 to 1.63; all but two are above 1.50. These densities are large compared with the known densities of solid peptides. Among the solid simple peptides only diglycine (1.71) and glycyl alanine (1.55) have such high densities. Solid polyglycine has a density of 1.51, but other solid synthetic polypeptides are much less dense: polyalanine, 1.28; poly γ -methyl L-glutamate, 1.32; poly γ -benzyl DL-glutamate, 1.28. Silk fibroin, a rather polar extended protein molecule, has a density of 1.36. Densities of about 1.50–1.55 for the molecules' outer regions are thus somewhat above what might be expected from the known densities of peptides, polypeptides, and dry proteins generally.

The polar and non-polar character of regions inside and outside R_{max} are compared for four proteins in Table 3, which shows the numbers of different types of atoms with distances from the centre of mass less than and greater than R_{max} . In these proteins, hydrophobic atoms are slightly more common in the interior than in the exterior. None of the $\phi_</\phi_>$ values can, however, be considered to indicate a tendency for the typical protein molecule interior to be markedly more hydrophobic than the exterior—a fact already evident from the work of Lee and Richards⁵. It is thus difficult to attribute the density discrepancy to a markedly different composition of the inner and outer portions of these protein molecules.

(c) Many water molecules may exist throughout the interiors of protein molecules. Column 12 of Table 1 shows the number of internal water molecules required to account for the difference between ρ_{calc} and ρ_{obs} . Numbers of the

TABLE 3 Numbers of different types of atoms present inside and outside of a sphere of radius R_{max} centred on the centre of mass

Protein	$R < R_{\text{max}}$				$R > R_{\text{max}}$						
	C_α	C_{hyd}	Polar atoms	Haem	C_α	C_{hyd}	Polar atoms	Haem	$\phi_<$	$\phi_>$	$\phi_</\phi_>$
Lysozyme	49	144	178	0	79	211	339	0	0.809	0.622	1.30
Myoglobin	48	173	148	34	108	334	446	10	1.399	0.771	1.81
Carboxypeptidase	162	538	583	0	145	409	599	0	0.923	0.682	1.35
Subtilisin	131	295	439	0	144	359	575	0	0.672	0.624	1.08

'Polar atoms' include oxygen, nitrogen and carbonyl carbons, ' C_α ' are the main chain α -carbon atoms, and ' C_{hyd} ' are all other carbon atoms—considered to be hydrophobic. $\phi_<$ and $\phi_>$ are the ratios of the numbers of ' C_{hyd} ' + 'Haem' atoms to 'polar atoms' for $R < R_{\text{max}}$ and $R > R_{\text{max}}$, respectively.

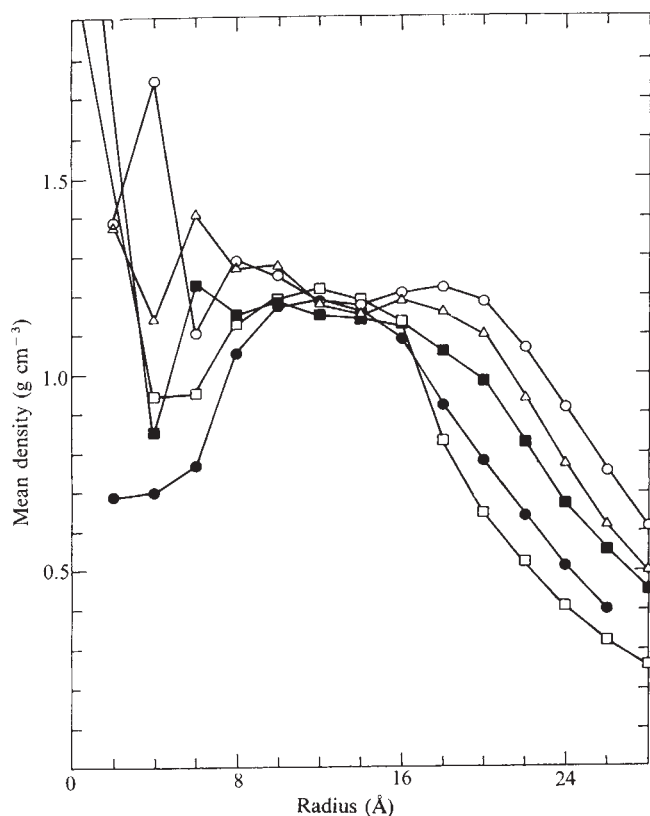


Fig. 2 Dependence of mean density of spherical regions on sphere radius. ○, carboxypeptidase; △, subtilisin; ■, chymotrypsin; ●, myoglobin; □, lysozyme.

order of 100–300 are indicated. Birktoft and Blow⁶ have detected approximately 16 localised water molecules within the chymotrypsin molecule. A much larger number than this is believed unlikely for proteins in general.

The second explanation (low density centre, higher density exterior) seems to be the most plausible, but contributions from all three factors are conceivable.

Atomic coordinates used in this study were supplied by the following: carboxypeptidase, W. Lipscomb; chymotrypsin, D. M. Blow; chymotrypsinogen, H. T. Wright; subtilisin, J. Kraut; lamprey haemoglobin, W. Love; lysozyme, D. Phillips; myoglobin, J. C. Kendrew; ribonuclease, F. M. Richards; staphylococcal nuclease, F. A. Cotton. The Princeton University Computer Centre facilities were used in making the calculations. The work was supported by a US National Science Foundation grant.

WALTER KAUFMANN
KEVIN MOORE
DIANA SCHULTZ

Department of Chemistry,
Princeton University,
Princeton, New Jersey 08540

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Control of Zea root elongation by light and the action of 3,5-diiodo-4-hydroxybenzoic acid

THE inhibitory effect of white light on the elongation of the primary roots of wheat and rice seedlings has been reported^{1–4}. It would seem that light affects the ability of the cells to increase in length and also reduces the meristematic activity of the root^{1,4}; the latter process may be dependent on the wavelength of the incident radiation⁴. Other studies here have shown that the chemical 3,5-diiodo-4-hydroxybenzoic acid (DIHB) can completely remove the inhibitory effect of light on the roots of rice and cress seedlings. This was achieved mainly by enhancement of cell elongation and not by stimulation of cell multiplication^{5,6}.

Following the discovery, in these laboratories, of the plant growth inhibitor xanthoxin (formed as a photo-oxidation product of certain xanthophyll epoxides⁷ known to occur in cereal roots^{8,9}) we decided to assess the possible role of the new inhibitor in the control of root elongation by light in relation to the action of DIHB.

Initial investigations were carried out to establish whether an exogenous application of xanthoxin affected the elongation of the primary roots of *Triticum* seedlings. Eclipse wheat seeds germinated for 20 h at 25°C in darkness to give a radicle length of 1 mm, were placed on Whatman No. 1 filter paper (5.5 cm diameter) in glass Petri dishes containing 2 ml of the appropriate test solution. Root length was measured after a 20 h incubation period at 25°C in white light (Mazda daylight fluorescent tube) or total darkness. Light

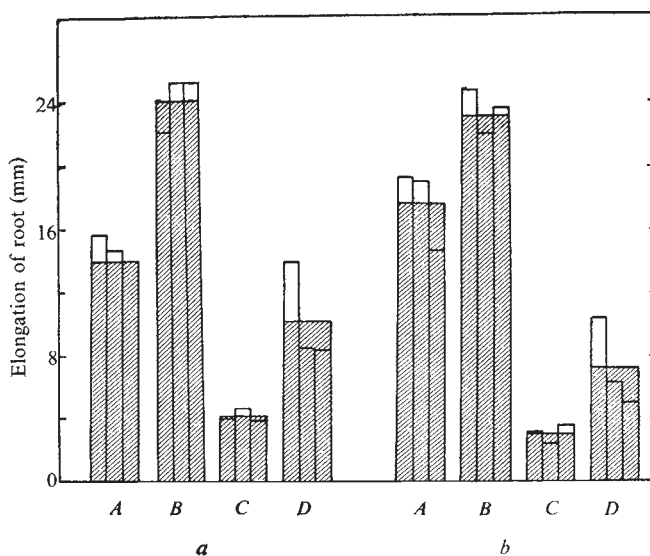


Fig. 1 Effect of 10 μ M xanthoxin and 0.1 mM DIHB solutions on the elongation of the primary roots of *Triticum* seedlings. Shaded areas are the mean of three independent experiments in which each treatment consisted of eight replicate seedlings. A, Control; B, DIHB; C, xanthoxin; D, xanthoxin and DIHB. a, light; b, dark.

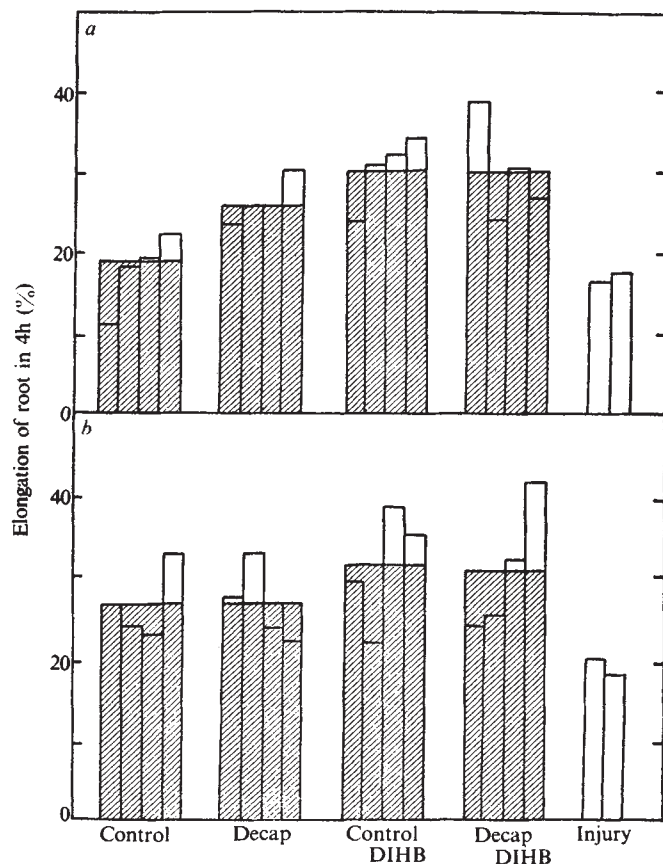


FIG. 2 Effect of root decapitation and 0.1 mM DIHB solution on the elongation of the roots of *Zea* seedlings exposed to white light or darkness. Shaded areas are the mean of four independent experiments in which each treatment consisted of eight replicate seedlings. *a*, light; *b*, dark.

exposure was found to induce a 21% reduction in the length of the wheat seedling roots. Application of a 10 μ M aqueous solution of xanthoxin resulted in an 83% reduction in elongation in the dark and a 72% reduction in the light compared with the water controls, (Fig. 1). Treatment with 0.1 mM DIHB solution gave a 56% increase in root elongation in the light and a 25% increase in darkness. A solution of 10 μ M xanthoxin, together with 0.1 mM DIHB, however, caused a 59% reduction of root elongation in the dark and only a 27% reduction in the light. In other words, DIHB seems to antagonise the inhibitory effect of xanthoxin on root elongation and this antagonism is more pronounced in the light than in the dark. An interaction of this type would be expected if xanthoxin was, in fact, involved in the endogenous inhibition of root elongation.

Further investigation⁹ revealed that xanthoxin could not be isolated from the roots of *Zea* seedlings 2-d-old exposed to white light. This finding was somewhat surprising as violaxanthin and neoxanthin were shown to be present in the light-exposed roots. The possibility still exists, however, that the rate of utilisation of the inhibitor might be so rapid that isolation of significant amounts would be difficult.

Microsurgical removal of the root cap of *Zea* has been shown to prevent the response of the roots to gravity¹⁰ and we have found that this treatment completely releases the roots from the inhibitory effect of light on elongation⁹. We decided, therefore, to determine whether the root growth-stimulatory properties of DIHB in the light were associated with this region of the root. Seedlings of *Zea mays* var. LG 11, 3-d-old and grown in darkness at 25° C on moistened Whatman No. 1 filter paper, were selected in green light for straight roots 20–30 mm long. Tests were carried out in glass Petri dishes 9 cm in diameter containing filter paper and

7 ml of the appropriate solution. Root length was measured immediately following treatment and again after a 4 h incubation period in white light or total darkness. Decapitated and control seedlings (with intact root apices) were placed in contact with distilled water or 0.1 mM DIHB solution. An additional control in which a 1 mm deep vertical incision was made into the root cap was used to establish the effect of injuring the root apex.

Roots of the water control increased in length by 18% and 27% during the 4 h incubation period in white light and darkness respectively, revealing again the inhibitory effect of light (Fig. 2). Decapitation of the roots of the seedlings exposed to white light resulted in an elongation equal to that of dark controls with intact apices⁹. Decapitation of the roots of the darkened seedlings, however, seemed to have no effect on root elongation. As the treatment designed to show the effect of injury to the root apex did not produce an enhancement of elongation, it was concluded that the root cap of *Zea* seedlings is implicated in the light-induced control of root elongation.

Application of DIHB to control and root-decapitated seedlings in the light and dark gave a uniform elongation of 31% to 32% during the 4 h incubation period. It is therefore clear that in presence of DIHB the decapitation had little effect on root elongation. Thus, it seems that the root growth-stimulatory properties of DIHB are independent of the light-induced control system which we have shown to be present in the root cap.

H. WILKINS
A. LARQUÉ-SAAVEDRA
R. L. WAIN

Agricultural Research Council,
Unit of Plant Growth Substances and Systemic Fungicides,
Wye College (University of London),
Ashford, Kent.

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Impact of cool temperatures on transformation of human and armadillo lymphocytes (*Dasypus novemcinctus*, Linn.) as related to leprosy

A CENTURY-LONG search for an unaltered animal in which to study leprosy in humans culminated in the finding that the nine-banded armadillo (*Dasypus novemcinctus*, Linn.), a primitive mammal native to the southern Western Hemisphere, develops disseminated lepromatous leprosy following inoculation with *Mycobacterium leprae* isolated from human tissue^{1–3}. Previous animals only developed leprosy following inoculation with leprosy bacilli after having been immunologically suppressed by neonatal thymectomy with or without irradiation of bone marrow^{4–8}. Yet curiously the leprosy lesions occurred chiefly on the cooler tissues, the footpads.

TABLE 1 Transformation of lymphocytes from 27 normal uninoculated armadillos to PHA at various temperatures.

	37° C		33° C		28° C	
	PHA	CT*	PHA	CT	PHA	CT
Mean	6,970	755	2,402	677	1,351	745
Standard deviation	1,908	196	1,707	196	491	178
Standard error of mean	367	38	329	38	263	34

Results expressed as an average value of duplicate cultures in c.p.m. of H-thymidine taken up by lymphoblasts.

* CT, control, no PHA added.

Binford and Brand noted that humans with leprosy were most severely infected in the cooler regions of their bodies such as the nose and digits, and so suggested that *M. leprae* may grow better at low temperatures^{9,10}. Storrs first suggested that the armadillo be used for the study of leprosy because its low body temperature (30° C to 35° C) was comparable with the cool regions of humans¹.

We reasoned that such a cool body temperature might depress metabolic activity and from this, cellular immunity of the armadillo might also be depressed and so enable infection by *M. leprae*. To test this we investigated the effect of temperature on the transformation of lymphocytes from armadillos when stimulated by phytohaemagglutinin (PHA) and lepromin.

The transformation of lymphocytes *in vitro* by mitogens is considered a measure of cellular immunity of the individual. More specifically, transformation of lymphocytes by mitogens may reflect the capacity of an individual to respond to and to control certain infectious agents, mycobacteria, brucellae, fungi and viruses, and to reject allografts and neoplasms.

Initially, lymphocytes from 27 normal, uninoculated armadillos were cultured with PHA following procedures previously described¹¹, with the exception that 10% foetal calf serum was used in place of autologous plasma. Armadillos were anaesthetised with ketamine hydrochloride (50 mg kg⁻¹) and 10–15 ml of blood were drawn by intracardiac puncture into heparinised syringes and transferred into siliconised tubes. Lymphocytes were separated and divided into nine equal samples and cultured in triplicate. PHA was added to two of the cultures in each triplicate set and no PHA was added to the third culture which served as a control. One of each triplicate set was cultured at 37° C, the second at 33° C and the third at 28° C, in a humidified atmosphere of 5% CO₂ for 72 h. Lymphocyte transformation was measured by the uptake of ³H-thymidine. A Packard Tricarb Scintillation Counter measured the results which were expressed as an average of duplicate samples in c.p.m.

The transformation of lymphocytes stimulated by PHA was reduced by approximately 66% when the lymphocytes were cultured at 33° C and by 81% when cultured at 28° C as compared with transformation of lymphocytes at 37° C (Table 1). Thus, a depression of lymphocyte responsiveness to PHA occurred at low temperatures or temperatures comparable with the body temperature of the armadillo.

Second, transformation of lymphocytes by lepromin was measured using lymphocytes from 13 armadillos. We used 0.1 ml of lepromin (containing 3×10^7 sonicated *M. leprae* ml⁻¹) as the specific mitogen, instead of the non-specific mitogen, PHA. The technique was otherwise identical to that used with PHA. Four of the 13 armadillos were uninoculated animals and seven were uninfected, but inoculated with *M. leprae* 30 months before the *in vitro* challenge of lymphocytes by lepromin. The remaining two armadillos which were inoculated with *M. leprae* were grossly infected with leprosy.

A marked reduction of transformation of lymphocytes by lepromin from all armadillos occurred at 33° C and 28° C when compared with transformation at 37° C. Lymphocytes

from uninoculated armadillos which were cultured at 37° C transformed at 5,621 c.p.m. (average) while the lymphocytes from armadillos inoculated but not grossly infected with *M. leprae* were more responsive, transforming at 14,505 c.p.m. (average). This increase in transformation of lymphocytes by lepromin reflects sensitisation of the lymphocytes resulting from inoculation with *M. leprae* 30 months previously. Furthermore, lymphocytes from the two inoculated armadillos with gross evidence of leprosy responded nearly identically to the uninoculated animals. The lymphocytes from the grossly infected armadillos averaged 5,680 c.p.m. This lack of an increase in the transformation response of lymphocytes from infected armadillos following stimulation by lepromin could be explained in several ways.

One possibility is the two infected armadillos were not sensitised following exposure to antigens which were acquired by inoculation with *M. leprae* and that the lack of sensitisation reflected the lack of resistance to *M. leprae* which led to lepromatous leprosy.

A second possibility is that the two infected armadillos were initially sensitised as a result of inoculation of *M. leprae* but later anergy developed similar to that occurring in humans with advanced lepromatous leprosy¹², due to a replacement of lymphoid tissues by invading *M. leprae*. But we are unable to explain why the lymphoblastic transformation response was comparable with that of normal armadillos and not more depressed.

Transformation of lymphocytes from humans was also studied to determine if cool temperatures would depress the transformation of lymphocytes. Heparinised blood (15 ml) was obtained by venepuncture in siliconised test tubes from nine healthy, young adult males and one woman. Three of the male subjects had been exposed to *M. leprae* while working for several years in an infectious diseases laboratory and the woman had worked for 5 yr in a

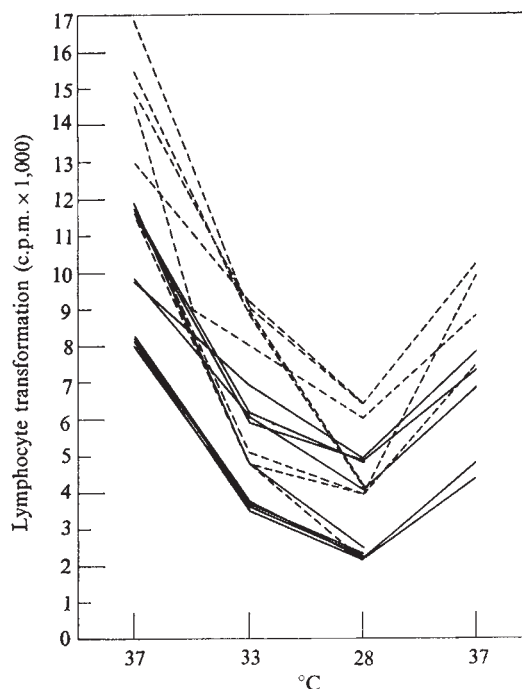


FIG. 1 The lymphoblastic transformation response of lymphocytes cultured at 37° C, 33° C, and 28° C when stimulated by PHA or lepromin is illustrated. An increase in transformation of lymphocytes is demonstrated to the right. The increase occurred when the lymphocytes were transferred from a 28° C incubator to a 37° C incubator and cultured for an additional 48 h. The c.p.m. is a measure of ³H-thymidine taken up by transforming lymphoblasts. The average value of the control (no PHA or lepromin) was 998 c.p.m. —, Phytohaemagglutinin; ---, lepromin.

leprosarium. The lymphocytes were cultured and stimulated by PHA and lepromin as had been done with lymphocytes from armadillos.

In all instances a depression of transformation of lymphocytes occurred at 33° C and 28° C (Fig. 1). The average value of the control, to which no mitogen was added, was 998 c.p.m. No increase in transformation by lepromin occurred in cultures of lymphocytes from the four human subjects who were previously exposed to *M. leprae* as compared with transformation of lymphocytes from six humans not exposed to *M. leprae*. This finding probably reflects non-sensitising exposure to antigens of *M. leprae* rather than an inability of the subject to become sensitised.

Lymphocytes which had been cultured for 72 h with PHA or lepromin at 28° C were transferred to an incubator at 37° C and cultured for an additional 48 h to determine whether the transformation response could be restored. The transformation response was restored by more than 50% (Fig. 1), thus directly implicating temperature as the agent responsible for the depression of the lymphocyte transformation.

We conclude that cool temperatures probably depress cell-mediated immunity *in vivo* and that such a depression of CMI could explain the susceptibility of armadillos to infection with *M. leprae*. Yet another possibility is that *M. leprae* multiplies best at cooler temperatures because a cool environment is optimal for their multiplication.

But, evidence to the contrary has been recently reported: more extensive multiplication of *M. leprae* *in vitro* has been reported in human macrophages at 37° C than at 33° C (ref. 13). If the optimal temperature for the growth of *M. leprae* is 37° C, then the armadillo's susceptibility to leprosy cannot be explained on this basis because its body temperature is much cooler than 37° C. Our finding of normal appearing thymolymphatic organs in the armadillo further supports that the cool body temperature of the armadillo is probably the significant condition responsible for the immune incompetence of the armadillo to *M. leprae* (D. T. P., G. P. W., E. E. Storrs and C. Gannon, unpublished).

The lepromatous leprosy that develops in the armadillo involves internal visceral organs and tissues that are near the body surface¹. The cool body temperature of 30° C to 35° C of the armadillo enables lesions to develop in viscera, whereas in humans lepromatous lesions are generally limited to superficial cool regions.

Cellular immunity may be modified by changes in temperature, for instance, an increase in body temperature could possibly enhance cellular immunity. The role of fever as a potentiator of cellular immunity against malignant neoplasms and infectious agents needs to be assessed. Furthermore, the impact of cool temperatures also needs to be assessed as related to susceptibility to other infectious agents.

DAVID T. PURTILO

Armed Forces Institute of Pathology,

GERALD P. WALSH
ELEANOR E. STORRS

Gulf South Research Institute,
PO Box 1177,
New Iberia, Louisiana 70561

ISAAC S. BANKS

Armed Forces Institute of Pathology,
Infectious Diseases Branch,
Washington, DC 20306

Received November 26, 1973.

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Die-back of *Pistia stratiotes* on Volta Lake, Ghana

ANNUAL die-back of *Pistia stratiotes* L., associated with an epidemic of an aphid-transmitted virus infection whose symptoms are leaf yellowing and brittleness, was recently reported for the Ibadan area of Nigeria¹. It was supposed that similar virus epidemics may have checked the initial outburst of the weed on the Volta Lake in Ghana. We confirm that *Pistia* has died back on the Volta Lake from a maximum cover of about 4% of the lake surface in 1966, 2 yr after the dam was closed, to about 1% now (1973). In recent work on the Pawmpawm arm of the lake we have measured the rate of retreat of *Pistia* mat from the main lake. By marking flooded trees near the edges of the mat, it was possible to survey the limits of *Pistia* in August 1971, April 1972 and in July 1973. These surveys, together with an aerial photograph taken in October 1968 (P. C. Pierce, personal communication) made possible the construction of the map shown in Fig. 1, from which it can be calculated that *Pistia* receded at an average rate of about 7 ha yr⁻¹ over the period of observation.

In 2 yr of regular observations², *Pistia* retreat resulted from disruption of *Pistia* mats by storms in the late dry season (February and March), following several months during which the plants, even in enclosed permanent plots, had been severely weakened by poor growth. Aphids were rare in the populations observed, and there was no evidence that virus disease was of primary importance in causing leaf yellowness or brittleness. Size and rapidity of fluctuation in lake-level and antagonism by other plants, which have been implicated in *Pistia* die-back in other areas^{3,4,5}, also seem to be unimportant on the Volta Lake. Retention time here is 4 yr as against 76 d for Lake Kainji, Nigeria³; in any case, the stranding of plants in the drawdown area

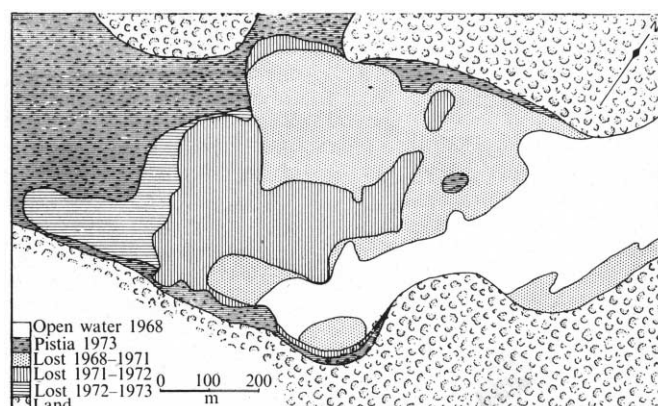


FIG. 1 Die-back of *Pistia stratiotes* on the Volta lake, Ghana.

should favour seed germination⁶ and is therefore unlikely to be responsible for disappearance of *Pistia*. The plant has not been replaced for the most part by any antagonising weed.

Circumstantial evidence suggests that poor growth of *Pistia* during the dry season may result from low nutrient status of the lake-water at this time, because of lack of rain to wash in nutrients from adjoining land. Lake nutrient status is probably improved during the early rains as a result of enhanced mineralisation by re-wetting of previously dry soil⁷, inflow to the lake of nutrients from such mineralisation and, possibly, direct enrichment of the lake by nutrients carried in early rainwater⁷. *Pistia* is known to respond sensitively to substrate nutrient level⁸, and especially to nitrogen⁴ the level of which increases many-fold as a result of re-wetting of dry tropical soils. Explosive growth of *Pistia* on the Volta Lake occurs following the first rains after the main dry season². There is evidence⁹ to show that nutrients such as phosphates have now dropped to less than half the level they reached when the lake was first formed.

The long-term disappearance of *Pistia* from the Volta Lake may be reflecting a change in the nutrient level, and probably also the decay and disintegration of flooded trees which originally helped to anchor *Pistia* mats.

D. U. U. OKALI
J. B. HALL

Department of Botany,
University of Ghana,
Legon

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Progress of a breeding project for non-human primates in Colombia

THE International Primatological Society¹ and several recent publications²⁻⁸ have pointed out the urgent need for conservation of non-human primates. The capture of live primates for exportation is a major drain on natural populations and breeding programmes are a vital need which has been emphasised by two recent events. First, the Institute for Development of Renewable Natural Resources in Colombia has established limits and standards for the hunting of non-human primates⁹. Second, a large scale, international research project has been launched through a grant from the US National Institutes of Health to the Institute of Laboratory Animal Resources of the US National Academy of Sciences, which will tackle critical questions of supply and demand, and seek to identify primates which must be bred to meet research requirements¹⁰. (In 1969, 16,295 New World primates and 42,514 Old World primates were sold in the United States solely for research³.) We report here preliminary results of the first attempt at large scale, controlled breeding of New World primates in the natural habitat of an exporting country, and discuss the feasibility of future projects.

In 1967 Mike Tsalickis, an animal dealer in Leticia, Amazonas, Colombia, initiated a breeding experiment with squirrel monkeys (*Saimiri sciureus*), the New World primate most widely used in research. (*Saimiri* constitutes 10–80% of primates exported to the United States from Latin America³.) Between 1967 and 1970, Tsalickis released 5,690 monkeys (1,200 males and 4,490 females, some judged to be pregnant) on Isla de Santa Sofia II which was previously uninhabited by non-human primates. (Three adjacent fluvial islands under Colombian jurisdiction called Santa Sofia are designated Santa Sofia I, II and III.) The monkeys could not leave the island without swimming at least 1 km, and hunting and trapping were prohibited. In 1971 Tsalickis, on the basis of a sample count of females with young, estimated the birth rate at 80%. Assuming no mortality, he calculated the existing population at 20,698^{11,12}.

Santa Sofia II is a 1,000 acre (400 hectare) island in the middle of the Amazon River 33 km upriver from Leticia, formed by fluvial deposition perhaps as recently as 40–50 yr ago. Lakes devoid of trees form one-third of the island which is uninhabited by monkeys. (The level of the river fluctuates nearly 7 m seasonally, more than 90% of the island may be inundated during the peak of the wet season in April and May.) Another third is formed by low areas and the edges of the island which are covered by a successional community including *Cecropia* sp. The remaining third of the island is formed by the central and higher areas which are covered by a more advanced successional stage with a mixed community of non-deciduous trees. About 10% of the island has been cleared for agricultural purposes, including fruit trees (banana and guava) planted for the breeding project. Several areas were sporadically provisioned with fresh fruits and monkey chow to supplement natural food available.

From June to early August of 1972 we conducted a census of the *Saimiri* population on the island. Our primary objectives were: (1) to measure the population as accurately as possible; (2) to make recommendations concerning collection and (or) other aspects of population management; and (3) to demonstrate that multidisciplinary efforts can enhance the value of field studies, and that for projects of this sort they may be essential. In this report we describe briefly our census methods and results and their implications. (We will publish a more detailed report later¹³. In addition a report of a subsequent project conducted independently by R. C. B. on the monkeys of Santa Sofia II is in preparation and will provide further data pertinent to the present discussion.)

Our census procedure was chosen to accommodate the topography, vegetation and seasonal flooding; the behaviour and ecology of *Saimiri*, and the availability of time and personnel. To measure the total population we needed to estimate (1) the number of groups on the island, and (2) average group size. We first divided the island into three workable sections (areas A, B and C), aided by the presence of natural ecological barriers such as permanent large ponds (Fig. 1). We then cut sixty-one trails, forty-five at 100 m intervals and sixteen at 200 m intervals, the distance between trails depending on type of vegetation and resultant local visibility. All our trails were perpendicular to and connected by three trails cut by Tsalickis, and facilitated access to most of the island land mass (Fig. 1). The number of groups of *Saimiri* was estimated by repetitions of a synchronic multi-strip census, whereby four to seven field workers walked blocks of adjacent trails, locating groups, until all the trails in an area had been covered in one continuous period. Data on group size, direction and rate of group movement, group cohesion and intergroup distance were also gathered by each observer to aid in determining average group size, and to avoid counting groups more than once. The average group size was estimated in two ways: (1) by averaging accurate

TABLE 1 Population estimates based on two approaches to group size

Area	Average group size	Average No. of groups	Extrapolation of population estimate
A	41	10	410 (in 42% of land mass)
B			287 (in 30% of land mass)
C			269 (in 28% of land mass)
Total			966

Area	Estimated group size	Average No. of groups	Population estimate
A	50	10	500
B	50	4	200
C	50	3	150
Total		17	850

counts of several groups in area A, the most accessible area; and (2) by using field observation from all three areas. The total population of the island was also estimated in two ways. (a) The population of area A was determined by multiplying its average group size (method (1)) by the total number of groups in that area. Assuming comparable density throughout the island (on the basis of similarity of habitat), we then extrapolated to areas B and C on the basis of land area. The three area populations were then added to obtain the total island population. (b) The total island population was obtained by multiplying the estimated average group size (method (2)) by the total number of groups in all three areas.

Table 1 shows the total island population estimates obtained by the two methods. Method (1) yielded a population of 410 for area A, and a total island population of 966. Method (2) yielded a total island population of 850. Areas A and B were each censused seven times under varying conditions (time of day, weather, composition of observing teams and direction of movement of observers). Area C was censused twice. Approximately 350 man hours were spent in direct censusing. The variability in number of groups sighted was low (s.d. = 1.13), indicating relative consistency and lending confidence to the method. (Continuing field research by R. C. B. on Santa Sofia II generally supports the number of resident groups estimated during the summer of 1972.) We therefore conclude that the *Saimiri* population of Isla de Santa Sofia II as of August 1972 was between 850 and 966. Based on variance in our counts (s.d. = 1.13), the probability that there were more than 1,200 or fewer than 550 monkey on the island is less than 0.0001.

Our results have two possible interpretations: (1) if published estimates are reliable¹¹ there was a decline from 20,698 to 850–966 in 18 months, or (2) previous estimates are unreliable. We are not aware of any unusual or altered conditions such as flooding or disease which might account for such a drastic mortality during the 18 month interval, nor

has Tsalickis indicated such conditions. Previous estimates of population size were based on too little evidence and even then were too optimistic because the calculations assumed no mortality. In either of the interpretations census figures must be viewed in relation to the documented number of animals introduced to the islands (5,690). Substantial decline is evident.

We can only speculate as to causes of mortality, particularly since there is no way of knowing when and over what period the decline occurred. Nevertheless, clearly many important aspects of *Saimiri* behaviour and ecology were not known or not taken into account when this breeding experiment was planned. Factors that need to be considered include abundance of food, spatial requirements, predator pressure, disease, climatic change, consequences of introducing alien groups of unfamiliar and unrelated individuals, and the effects of a continued influx of new animals on established groups.

M. Tsalickis has communicated his personal conviction that large numbers of monkeys were stolen from the island for sale in Leticia. The only evidence of poaching of which we are aware was reported to us by T. Jerkins of Tarpon Zoo, Tarpon Springs, Florida. In October 1972 an unspecified number of monkey traps were discovered on the island by Tsalickis' employees. On February 26, 1973 a tattooed monkey from the island was discovered in a shipment to the zoo. This is evidently one of the animals introduced to the island when only a small portion were tattooed. (No tattooed monkeys were found in the 1972 census and the subsequent study by R. C. B.) On the basis of present information, we consider the poaching hypothesis both unverified and unlikely, although it cannot be excluded as a possibility.

The single most important lesson to be learned from the Santa Sofia experiment is that controlled breeding of non-human primates on a large scale cannot be accomplished without considerable basic biological data to support careful planning and management—even when such projects are conducted in apparently representative habitat within the natural range of a species. The successful breeding of *Saimiri* in the enriched tropical hammock of the Monkey Jungle, Miami, Florida, has indicated that controlled breeding of certain non-human primates in a natural or seminatural environment is possible, when careful management, including daily dietary supplementation, is provided. Although they live in only a 4 acre area, the squirrel monkeys of Monkey Jungle increased from thirty-seven animals to approximately 150 in 6 yr. The original thirty-seven were handpicked and in apparent good health. The population was fed seven times a day, 7 d a week, to supplement the natural diet¹⁴. In this connection it is important to note that Monkey Jungle is a commercial zoo and not a primate ranch. For that reason, and in spite of its success, it cannot answer unequivocally the question of whether or not *Saimiri*, or any other species, can be bred economically, that is, at prices competitive with current sources of non-human primates.

The *Saimiri* breeding project of M. Tsalickis on Santa Sofia II is provisionally licensed as a Zoo-Criadero (faunal breeding farm) by the Division of National Parks of INDERENA. Tsalickis' effort has paved the way for future primate ranching attempts, and continued studies on Santa Sofia will help determine more of the critical factors in *Saimiri* husbandry. Finally, the Santa Sofia experiment has again emphasised the practical need for more data on the behaviour and ecology of non-human primates. Such data are essential for the economical, large scale husbandry which must be achieved if many species are to avoid depletion in nature and are to be preserved as a renewable natural resource.

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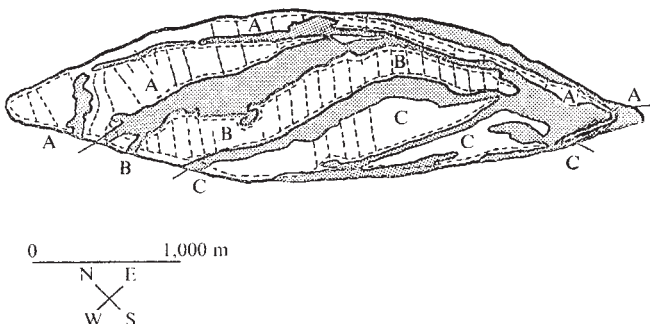


FIG. 1 Map of Isla de Santa Sofia II showing major survey areas (A, B, C), trails (dotted lines), and open water or marshy areas generally not inhabited by *Saimiri* (stippled).

and local housing and transportation. Financial support was also provided by the National Institutes of Health (to L.E.S.), Cornell University (to L.E.S. and D.S.B.), the US National Science Foundation (to R.A.M.), the Hooton Fund (to R.A.M.), and the Hunsaker Foundation (to R.C.B.). We also thank J. I. Hernandez, R. W. Cooper, and D. Hunsaker II of INDERENA for their help.

ROBERT C. BAILEY

Leticia, Amazonas, Colombia

RALPH S. BAKER

PO Box 41,
Brooklyn, Connecticut 06234

DOROTHY SLATER BROWN

Department of Psychology,
Cornell University,
Ithaca, New York 14850

PATRICK VON HILDEBRAND

University of the Andes,
Bogota, Colombia

RUSSELL A. MITTERMEIER

Department of Anthropology and
Museum of Comparative Zoology,
Harvard University,
Cambridge, Massachusetts 02138

LESLIE E. SPONSEL

Department of Anthropology,
Cornell University
Ithaca, New York 14850

KATHERINE E. WOLF

Department of Anthropology,
Brandeis University,
Waltham, Massachusetts 02154

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significance. In this way, a large sector of potential sensory input is 'blocked out'. He therefore conceived this function as a stimulus barrier; 'a special integument or membrane which keeps out the stimuli'. Relative failure of this 'barrier' is unpleasant, and part of common experience. Irrelevant sounds intrude into consciousness, and are irritating.

This 'barrier' can be conceived in terms of neurophysiological models for the habituation of the orienting response⁴. The orienting response consists of a number of behavioural and physiological changes induced in the subject by a novel stimulus. This response diminishes and ultimately ceases after a number of repetitions of the stimulus. The individual is then said to have habituated to that particular stimulus. A new stimulus re-evokes the orienting response, causing dishabituation. It is not unreasonable to suppose that the phenomenon of habituation reflects an individual's ability to 'block out', or to selectively not attend to irrelevant stimuli. The possibility that smoking may increase the effectiveness of a 'stimulus barrier' was examined by a study of the habituation of the orienting response in smokers.

The subjects were 10 young men (20–25 yr of age) who habitually smoked at least one packet of cigarettes a day. Alpha-desynchronisation of the electroencephalograph was the aspect of the orienting response chosen for study, since the peripheral effects of nicotine may have altered the galvanic skin response, which is more usually chosen.

The EEG was recorded in a sound-damped room. An active silver-silver chloride electrode was set at the vertex (cz on the international 10/20 system) and referenced to the left mastoid. The subjects were tested on four occasions at three week intervals. The first session involved familiarisation with the recording procedure. Two series of stimuli were presented through loud-speakers 2 feet from the subjects. The first series consisted of twenty loud (90 dB) brief (0.5 s) bursts of pure tone (2000 Hz) with interstimulus intervals varying between 30–80 s. The second series differed only in that the frequency of the tones was 1000 Hz. Each series ended with a dishabituating tone of 10 s duration. The habituation point was defined as the number of stimuli at which the EEG showed no desynchronisation to a stimulus, when there was also no desynchronisation to the two succeeding stimuli.

The percentage of alpha activity was determined between the fifteenth and sixteenth stimuli, and done after the recording session by one observer who was unaware of the smoking conditions. Skin resistances were recorded as described elsewhere⁵, and the heart rates taken by means of cardiometer coupler (9857) on an Offner-Beckmann dynograph.

Before the second session, subjects were instructed to smoke according to their usual pattern. The 2000 Hz series of tones was presented while the EEG was recorded. Forty-five minutes later, subjects were instructed to smoke two cigarettes of their preferred brand and were then presented with the 1000 Hz series of tones. The mean duration between the last cigarette smoked before the session and the onset of recording was 31.1 min, s.e. 3.1, with a range of 20–50 min.

Before the third session, subjects were instructed to abstain from cigarettes for 12 h. The two series of tones were again presented, one before smoking, and the second after smoking two cigarettes. The fourth session was identical to the third except for the substitution of a nicotine-free placebo cigarette (roasted chicory leaves in authentic cigarette packing) instead of the preferred brand. The subjects were told the cigarettes were 'foreign', and none of them realised that they were not smoking tobacco.

The results (see Table 1) indicated that the immediate effect of tobacco smoking was to greatly increase the speed of habituation, and that placebo smoking had no effect.

Thus, in the second session, the mean habituation point changed from 16.1–4.5, after smoking (paired $t = 7.74$,

Tobacco smoking and a 'stimulus barrier'

ONLY one in four smokers who wish to stop, actually succeed¹. The strength of the habit is hard to explain in terms of learning theory, since its manner of reinforcement is uncertain. This study considers a possible mechanism through which smoking might be positively reinforced².

Freud³ described a perceptual function which enables us to select from an overwhelming mass of sensory data only those aspects of the environment which have meaning or

TABLE 1 Means and standard errors for habituation point, heart rate, skin conductance and proportion of alpha

		Session 1 (Training, series 1)	Session 2		Session 3		Session 4	
		Non-deprived	Non-deprived	Non-deprived + cigarette	Deprived	Deprived + cigarette	Deprived	Deprived + placebo
Habituation point	Mean	16.9	16.1	4.5	13.8	5.6	14.7	13.7
	s.e.	1.5	1.6	1.0	1.5	1.3	1.7	1.8
	n*	8	10	10	10	10	10	10
Heart rate (beats/min)	Mean	74.4	70.4	72.0	61.6	72.9	59.5	57.8
	s.e.	4.3	3.7	3.3	4.0	4.3	3.6	4.6
	n	9	9	8	10	10	8	8
Skin conductance $\mu\text{mho} \times 10$	Mean	85.9	104.4	136.3	75.4	122.7	103.4	106.6
	s.e.	23.8	33.9	27.8	22.6	79.2	22.8	36.6
	n	8	10	10	10	10	10	10
% Alpha	Mean	21.6	23.2	30.0	28.4	34.5	26.5	27.9
	s.e.	6.6	4.4	3.8	9.1	7.1	6.8	5.0
	n	8	10	10	10	10	10	10

* Although all 10 subjects participated in all tests, some readings had to be discarded due to recording problems.

$P < 0.001$) and in the third session, when the subject smoked after 12 h deprivation, the mean habituation point changed from 13.8–5.6 (paired $t = 4.62$, $P < 0.005$). On the other hand, during the placebo smoking session the mean habituation point remained virtually unchanged 14.7–13.7 (paired $t = 0.92$).

Delayed consequences of smoking were not manifest in habituation rate since there was no difference between the rates after a period of normal smoking and after a 12 h deprivation. The mean habituation point before smoking in the second session was 16.1, while the mean habituation point after deprivation was 14.3 (paired $t = 1.14$).

Occasional failures to show a dishabituating response were evenly distributed between tobacco-smoking and placebo-smoking sessions.

Since the change in habituation was not found with placebo, it seems likely that the effect was mediated by a drug, presumably nicotine, rather than by psychological factors. Increase in speed of habituation usually reflects diminished arousal, and drugs which effect such a change are therefore considered to be sedative⁶. Nevertheless, the immediate effects of smoking on habituation cannot be adequately explained in terms of sedation, since this is not reflected in measures of arousal.

Changes in peripheral measures of arousal suggested that smoking may cause mild central nervous system excitation rather than sedation. The skin conductance tended to rise, while a significant increase was shown in heart rate during session 3 (paired $t = 5.52$, $P < 0.001$). This increase was not apparent in session 2 (paired $t = 0.90$). There was also a significant difference between the heart-rates of deprived and non-deprived subjects before smoking (paired $t = 4.87$, $P < 0.001$). These changes, however, may be purely autonomic and not be correlated with cerebral events. Nevertheless, they are consistent with certain previous electroencephalographic evidence^{7–10}, although in the present study no significant change in proportion of alpha activity was induced by smoking (paired t in session 2 = 1.27, and in session 3, $t = 0.69$).

Theoretical models of the habituation phenomenon suggest that it depends upon active inhibitory mechanisms⁴, of which a number have been described^{11–15}. Clinical studies involving habituation and measures which are likely to reflect central nervous system excitation, suggest an inverse relationship between 'inhibitory' and 'excitatory' processes¹⁶. It therefore seems not impossible that tobacco smoking causes a dislocation of the usual relationship, so that inhibitory mechanisms are stimulated without simultaneous reduction in CNS excitation.

In conclusion, the findings suggest a possible means of positive reinforcement of tobacco smoking through its effect upon the individual's manner of screening of sensory input.

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JEANETTE FRIEDMAN
THOMAS HORVATH
RUSSELL MEARES

Department of Psychiatry,
University of Melbourne,
Austin Hospital,
Heidelberg,
Victoria

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Reaction of methyl mercury with plasmalogens suggests a mechanism for neurotoxicity of metal-alkyls

THE biosynthesis of methyl mercury from mercuric ion, by microorganisms which live in sediments polluted with mercury, is of special significance to the bio-accumulation of mercury^{1–3}. Although the mechanism of methyl mercury biosynthesis is understood to a large extent at the molecular level^{4–6}, very little is known about the mechanisms of molecular interaction in both the bio-accumulation and the neurotoxicity of this compound. It is clear that ingestion by humans of food contaminated by methyl mercury leads to

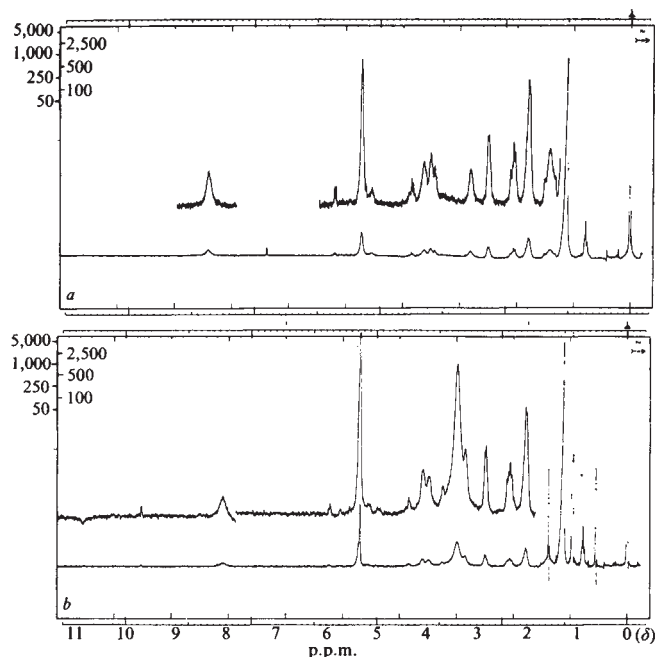


FIG. 1 *a*, 220 MHz NMR spectrum of plasmalogen. *b*, 220 MHz NMR spectrum of the products formed when plasmalogen is incubated with methyl mercury chloride and water.

the synthesis of methyl mercury chloride in the stomach, and this, being very non-polar, is easily transported into the blood stream⁷. The distribution of methyl mercury in animals is characteristically different from that of inorganic mercury, in that alkyl mercury compounds have a tendency to partition into the lipids, or hydrophobic regions of the cell⁸.

Post mortem examinations of victims of the Minamata disaster, who suffered neurological disease after eating fish contaminated with mercury, revealed extensive damage to the central nervous system. Widespread lysis of cell membranes, especially of neuroglia and granule cells, was reported^{9,10}. Methyl mercury in aqueous systems is an unreactive molecule, and it is hard to see how it could cause such irreversible damage. Although methyl mercury reacts readily with sulphydryl groups, it does not form stable complexes with nitrogen-containing basis in aqueous solution; in fact it does not complex with pyridine in aqueous solution¹¹.

It is possible that the reaction of methyl mercury with cysteine residues, which are present at the active sites of several important enzymes of the glycolytic pathway, could provide a basis for the neurotoxicity of this compound¹². Concentrations of methyl mercury greater than catalytic concentrations would have to be present, however, to be effective in enzyme inhibition. As the binding of methyl mercury to thiols is reversible, relatively large concentrations of methyl mercury would be required for that enzyme inhibition. Furthermore, inhibition of the enzymes in the glycolytic pathway would have an indirect and reversible effect on membrane synthesis. We report here that methyl mercury can react both catalytically and directly with a

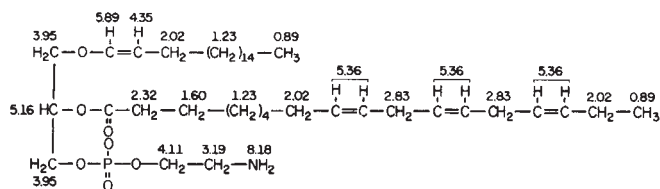


FIG. 2 Structure of plasmalogen with 220 MHz NMR proton assignments in δ (p.p.m.) adjacent to each carbon atom.

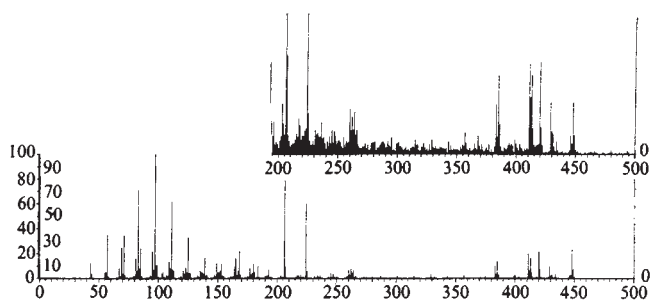


FIG. 3 High resolution mass spectra of the 2,4-dinitrophenylhydrazone derivatives isolated from the reaction products of the methyl mercury-catalysed hydrolysis of plasmalogen. Spectra were recorded at 10 eV and at 70 eV (inset).

group of phospholipids which are important in membrane structure for cells of the central nervous system.

High resolution nuclear magnetic resonance (NMR) has been used to assign all the protons of a plasmalogen (L α phosphatidyl ethanolamine), which has palmitic and stearic acid as alternative residues to the vinyl ether linkage at the α carbon, and linolenic as the unsaturated fatty acid at the β carbon. Methyl mercury chloride is soluble in this phospholipid, and the methyl mercuric ion catalyses rapid hydration and hydrolysis of the vinyl ether linkage to give a mixture of palmitic and stearic aldehydes plus the linolenic monoglyceride product. The course of this reaction was followed by 220 MHz NMR, and the aldehyde products were characterized further by mass spectrometry of their 2,4-dinitrophenylhydrazone derivatives. The relevance of this reaction to the neurotoxicity of metal-alkyls is probably linked with membrane lysis, which is specific for those membranes which contain plasmalogens as a major constituent of the phospholipid backbone in membrane structure.

Reaction mixtures were set up containing 24.1 mg of plasmalogen (phosphatidyl ethanolamine obtained from P-L Biochemicals) dissolved in 1.0 ml of CD_2Cl_2 to which 163.5 mg of methyl mercury chloride was added. When 0.17 ml of water was added the reaction proceeds at 25°C to yield palmitic and stearic aldehydes. We used a concentration of methyl mercury chloride twenty times greater than that of plasmalogen, but the catalytic nature of the reaction makes it possible to use much less. Decreasing the concentration decreases the rate of catalysis without altering the stoichiometry for the reaction.

Fig. 1 shows the 220 MHz NMR spectra of the plasmalogen before and after addition of methyl mercury chloride. From the spectrum in Fig. 1*a* it is possible to assign all the protons in this mixture of compounds. Fig. 2 shows the basic structure of the plasmalogen with resonances assigned in δ (p.p.m.); using tetramethylsilyl as an internal standard. The spectrum in Fig. 1*a* was integrated and the plasmalogen structure confirmed by these NMR data. Resonances of special interest in the overall reaction with methyl mercury chloride are those due to the protons of the *cis* double bond of the vinyl ether linkage which are assigned at $\delta = 4.35$ and $\delta = 5.89$ respectively (Figs 1*a* and 2). Also the resonance at $\delta = 8.18$ which represents the protons of the primary amine of ethanolamine is interesting because this resonance shifts from $\delta = 8.18$ to 8.07. This shift is indicative of a weak interaction between methyl mercury and this basic amino group; an interaction which does not occur in aqueous solution. Therefore, the hydrophobic nature of the phospholipid facilitates coordination to nitrogenous bases to some extent. When methyl mercury chloride is added to the plasmalogen, the vinyl ether resonances at $\delta = 4.35$ and $\delta = 5.89$ decrease in intensity and a new resonance which is characteristic of the presence of a long chain aliphatic aldehyde appears at $\delta = 9.69$ (Fig. 1*b*).

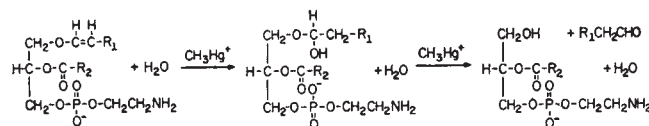


FIG. 4 Proposed reaction sequence for methyl mercury-catalysed hydrolysis of plasmalogen.

The aldehyde products formed in this reaction were characterised as their 2,4-dinitrophenylhydrazone derivatives. The reaction products from the NMR experiment were transferred to a reaction vessel and allowed to react with 1.0 ml of 0.2% 2,4-dinitrophenylhydrazine in 2N H_2SO_4 . The reaction was allowed to proceed for 1.5 h and then the products were extracted into chloroform. The chloroform layer was dried over anhydrous sodium sulphate and evaporated to dryness. The yellow residue was dissolved in 0.5 ml of benzene and applied to a silica gel column (2 cm $\times$ 17 cm). The column was eluted with benzene, and 5.0 ml fractions were collected. Two yellow compounds were eluted from the column, and the first fraction to elute was evaporated to dryness and subjected to mass spectrometry (Fig. 3). Molecular ions at m/e 420 and m/e 448 are consistent with the product being a mixture of palmitic and stearic aldehyde 2,4-dinitrophenylhydrazones. The fragments at m/e 385 and m/e 413 are due to the loss of H_2O and OH from the palmitic and stearic derivatives respectively. The peaks at m/e 224 and 206 are due to McLafferty rearrangements involving three carbons of each aldehyde chain, and the 2,4-dinitrophenylhydrazone portion of each derivative¹³. The rest of the fragmentation pattern is consistent with that of a mixture of C_{18} and C_{16} saturated aliphatic carbon chains.

High resolution NMR is especially valuable for the study of the structure and reactivity of phospholipids in solution because all the protons for these complex molecules can be assigned. We have shown that methyl mercury reacts catalytically in promoting hydration and hydrolytic cleavage of the vinyl ether linkage in plasmalogens to give a mixture of long chain saturated aldehydes. A sequence for this reaction is proposed in Fig. 4. The fact that methyl mercury is soluble in phospholipids, and causes lysis of certain cell membranes in the central nervous system makes it tempting to speculate that this reaction could have significance in the neurotoxicity of this metal-alkyl. Also, it is expected that other metal-alkyls should catalyse this reaction by a similar reaction sequence.

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H. J. SEGALL
J. M. WOOD

Department of Biochemistry,
Roger Adams Laboratory,
School of Chemical Sciences,
University of Illinois,
Urbana, Illinois 61801

Received November 23, 1973.

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GENERAL

Hydrogen fuel

HYDROGEN is receiving much attention as a clean fuel¹⁻⁴. It is usually stated or implied that hydrogen is a clean fuel because only water is produced when hydrogen is ignited in an engine. Unfortunately, this is not a fact. The following reaction also occurs:



Furthermore, as energy is extracted from the reaction as work, the reaction favours the production of more hydrogen peroxide.

A one-cylinder Briggs and Stratton engine was equipped with a propane-type carburettor, and the muffler was replaced with a stainless steel water-cooled condenser 3 feet in length. A dual feed system enabled it to be started using propane. When the engine had reached 1,750 r.p.m. and was running smoothly, the fuel was slowly changed from propane to hydrogen while the engine speed was maintained. After the engine had been running on pure hydrogen for 10 min the condensate from the exhaust was collected and analysed for H_2O_2 .

Qualitative tests with $\text{TiOSO}_4\text{-H}_2\text{SO}_4$ solutions and with Tes Tape urine sugar analysis paper indicated a strong positive for H_2O_2 . The concentration was analysed using a standard KMnO_4 titration. The results of three separate runs are given in Table 1.

TABLE 1 Concentration of H_2O_2 in the exhaust of an engine using H_2 gas as a fuel

Test No.	H_2O_2 in the exhaust (p.p.m.)
1	230
2	220
3	220

Although the concentration of H_2O_2 in the exhaust was not high in these experiments, the implications of using a large number of big engines producing H_2O_2 in the environment must be considered. Hydrogen peroxide is known to generate free radicals which would be a potential hazard if inhaled. If they are to be completely safe, engines operating on hydrogen gas will probably require some type of catalytic converter for the exhaust gases.

E. J. GRIFFITH

Monsanto Industrial Chemicals Company,
800 N. Lindbergh Boulevard,
St Louis,
Missouri 63166

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book reviews

Men from beyond Mars

Communication with Extraterrestrial Intelligence. Edited by Carl Sagan. Pp. xx+428. (MIT: Cambridge, Mass. and London, November 1973.) \$10.

IN sight of Mount Ararat, reputed resting place of Noah's Ark, scientists from six countries met to confer on "Communication with Extraterrestrial Intelligence" (CETI) in September 1971. The 54 delegates concluded: "It seems to us appropriate that the search for extraterrestrial intelligence should be made by representatives of the whole of mankind".

This volume is a transcript of the conference, which was held at the Byurakan Astrophysical Observatory in Soviet Armenia. The observatory's director, Viktor Ambartsumian, was chairman of the Soviet delegation.

The first-ever CETI conference was an unofficial gathering at Green Bank, USA, in 1961, at which Frank Drake presented the now classic formula for calculating the probability of communicative life in the Galaxy, which has since been discussed and elaborated in various papers and books. More than half the Byurakan proceedings consist of a wide reassessment of the many implications in the formation of planets, origin of life, rise of intelligent life and the desire to communicate. Contributors to the debate include Francis Crick, Tom Gold, V. I. Moroz, Leslie Orgel and Y. N. Pariisky.

Surprisingly, the Byurakan estimate differs little from previous suggestions. "We have estimated the rate of formation of such civilisations in the Galaxy: one is formed about every 10 years", says Carl Sagan, the United States delegation's head, in summary of the debate.

Yet the problem of estimating the lifetime of such a civilisation remains the greatest unknown in any such calculation: "If civilizations destroy themselves shortly after arising, there may be no one for us to talk to but ourselves", says Sagan. Canadian anthropologist Richard Lee argues for the positive effect of CETI on a civilisation: "If we receive a communication we want to stick around to see what the reply is".

"There are a million technical civilisations in the Galaxy. This corresponds roughly to one out of every hundred thousand stars", Sagan concludes cautiously on page 166, freely admitting the possible massive error in this figure. A

few pages on, Sebastian von Hoerner makes his own estimate—600 light years—of the distance between current communicative civilisations.

Startling news to come later in the conference was V. S. Troitsky's revelation (page 256) that the Andromeda galaxy and 11 stars near to Earth, including Tau Ceti and Epsilon Eridani, had been monitored in autumn 1968 for artificial signals. This was the first attempt since Drake's project Ozma to eavesdrop on stars. A proof note is added to outline the recent similar work of G. Verschuur (*Icarus*, 329; 1973) and another United States project. All such projects have had negative results.

Recently, N. S. Kardashev and Troitsky have reported possible artificial signals from space, breaking Iosef Shklovsky's rule of thumb presented at Byurakan: "We must follow the legal principle of presuming signals natural until proved otherwise".

Several papers have already been spun off from discussions and presentations at the CETI conference, including one by Sagan (*Icarus*, 350; 1973) which is reprinted as an appendix. Also previously published are Freeman Dyson's free-ranging presentation to Birkbeck College in 1972, *The World, The Flesh and The Devil*, and the CETI conference resolutions (*Icarus*, 412; 1972).

Another topic discussed at Byurakan was astroengineering—large-scale projects of advanced civilisations that might produce tell-tale signs such as extensive amounts of infrared emission. Techniques of contact with extraterrestrials were outlined, with Bernard Oliver summarising the project Cyclops report (NASA CR 114445). Should such an expensive undertaking be funded? Oliver is in no doubt: "All past human history may indeed be merely a prelude to an inconceivably exciting future as participants in a galactic culture".

Linguist B. V. Sukhotin reveals the development of a decoding programme, in a short section on the possible contents of extraterrestrial messages. Then follows a brief discussion involving historian William H. McNeill on the consequences of such contact. "We need from the beginning to accept responsibility for the effect of what we may be planning", says Philip Morrison. Sagan and Morrison have since taken part in another discussion on ETI with participants including a theologian, a philosophical viewpoint not represented at Byurakan.

The conference resolutions outline a number of directions for future research, and suggest new radio and infrared instruments to aid the search. (The Soviet 600 m radio telescope to be opened soon will be partly used for CETI research.) "All of the instruments described above have the capability of providing important data in subjects quite separate from CETI", the participants point out.

But it was Shklovskii who summed up their main problem: "All the natural sciences rely upon observations and experiments. We have none of that here".

IAN RIDPATH

School to college

Continuity and Discontinuity: Higher Education and the Schools. Pp. xi+116. (A Report and Recommendations by the Carnegie Commission on Higher Education, August 1973.) (McGraw-Hill: New York and London, October 1973.) £2.95.

REPORTS on education are not, as a rule, noted for their attractive reading. This one is an exception, partly on account of its stimulating and imaginative mode of presentation, and partly because of the fascinating light it throws on some of the more intransigent educational problems that face us in Britain today. It is sometimes comforting to see one's own difficulties painted larger than life in the colours of another country. Chapters 2 and 3 of the report provide a condensed history of American educational evolution dating from 1850 and projected into the year 2000. Some outcomes are an emphasis on the need for more closely coordinated admissions policies from school to college, the need to experiment with D. Arts degrees as teaching alternatives to the PhD, and the necessity for revised and more standardised testing procedures. Chapters follow on the reform of the admissions procedure to college, improvements in secondary education, new structural patterns relating to such factors as course content, the education of school teachers and administrators, and possible mechanisms for better school-college collaboration. Chapter 1, being a statement of major themes and recommendations, seems somewhat out of place and would have been better at the end as an outcome of all that had gone before.

Several sections of the report are of particular interest at the present time:

for instance, a scheme for shortening the length of the college course to produce a BA qualification somewhat similar to our proposed Dip.HE. It is interesting to be told that teacher education is now regarded as the largest single enterprise in American higher education. Clearly, many of the problems in this field, with the teacher training curriculum, teaching practice arrangements in schools, and in-service training closely parallel our own.

The report will thus be of interest to anyone concerned with the planning of secondary or higher education. Reading it, I could not help but feel profoundly thankful that Britain is a relatively small nation with a public examination system and entry requirements to higher education subject to some degree of central control and hence an equivalence of standards.

W. H. DOWDESWELL

Glimmer of hope

Priorities for Action: Final Report of the Carnegie Commission on Higher Education, with Technical Notes and Appendixes. Pp. x+243. (McGraw-Hill: New York and London, October 1973.) \$4.95.

THE ability of the British to look into their future by looking into the American present has been disturbed by the advent of instant communication, through which the American present influences not only their future but also their present. They therefore face the paradoxical situation that while in the sphere of mass higher education they have not yet caught up with the United States of the 1950s, they share in the disillusionment and graduate employment problems of the 1970s. "A traumatic loss of sense of assured progress has occurred (page 6) the world over, and as a result the British are in danger of accepting their inferiority to the educationally developed nations permanently and disastrously. The report, with its dispassionately balanced outlook, provides an effective antidote to this policy of despair.

"In 1870, the issue was the modernisation of higher education—how to adjust to industrial and agricultural expansion, to political populism, to the rise of science. To-day the issue is more the humanisation of higher education—how to respond to the greater aspirations of more individuals for a higher quality of life, how to adjust to the social facts of more affluence and more leisure, how to incorporate into higher education the rise of the creative arts." (page 21.)

"One of the interesting—and, to some, surprising—discoveries of recent times has been that quality and quantity are

not necessarily inconsistent with each other in the academic world". (page 27.)

"We have suggested special admission provisions for disadvantaged students where their ability and the special assistance of the College will make possible their meeting, in full, the academic standards of the college within a reasonable period of time and certainly by graduation. Remedial work will be necessary." (page 37.)

"Planning for the future of higher education should be on a contingent basis, subject to constant re-examination. . . . The technocratic planning analysts are bound to be wrong." (page 80.)

Finally and rightly, the report quotes Ashby's remark about the need to preserve the "thin clear stream of excellence" (page 30). But are we always sure that in pretending to protect excellence we are not protecting privilege?

The editor has restricted me to 400 words, and within that compass I cannot do justice to this remarkable report. The purpose of the disconnected quotations above is to persuade politicians and civil servants, industrialists and businessmen, academics and students, to read this remarkable report whole and to take heart from it.

L. R. B. ELTON

Pacific problems

Nature Conservation in the Pacific. Edited by A. B. Costin and R. G. Groves. Pp. xvi + 337. (Proceedings of Symposium on Nature Conservation in the Pacific of 12th Pacific Science Congress held in Canberra, August–September 1971.) (Australian National University Press: Canberra, April 1973.) \$12.50.

LIKE most other conference proceedings, this book has taken an unconscionable time to produce. Yet since Dr Lee Talbot says on page 261 that "relatively little progress on conservation problems has occurred in the South-east Asian region" since the Bangkok Conference of the International Union for the Conservation of Nature in 1965, perhaps this does not matter much. Nature conservation problems seem to be either static or dynamic in the wrong direction. For Dr Talbot goes on to add that most of the same problems that existed in 1965 are still problems, some of them considerably aggravated, while a whole range of new problems have appeared.

Although the subjects covered by the symposium are wide ranging, the eastern Pacific is almost wholly omitted, the northern Pacific is grossly underplayed, and Australia and its neighbouring region bulk as large as one would expect from the locale.

Many of these papers are excellent, but I wonder whether it is useful to the scientific community to have them all

printed, after an undue delay, in book form? Would it not be better to have an abstracting service for these conferences and symposia, widely circulated soon after the event, and enabling people interested in particular papers rapidly to get a copy?

RICHARD FITTER

Drug receptors

A Guide to Molecular Pharmacology-Toxicology. Edited by R. M. Featherstone. Part 1: pp. xiv+425; part 2: pp. xiii+426–811. (Modern Pharmacology: 4 series of Monographs and Textbooks, vol. 2.) (Dekker: New York, September and November 1973.) Part 1: \$29.50; part 2: \$18.75.

MOLECULAR biology, it is said, owes its origins in no small part to the application of novel physical and chemical techniques to biological problems. These books are intended to go one step further, by describing how several of the newer techniques of molecular biology are being used to solve interesting and important problems in pharmacology and toxicology. They contain a collection of 21 chapters by a total of 30 authors, each chapter devoted to model systems, methodology, or both. As the editor says, there is no great thread of continuity among the chapters and none was intended. Nevertheless, the books are aimed pretty squarely at proteins as drug receptors: witness the contorted sausage on the dust jacket and the categorical statement on page 308 that "whenever a drug receptor has been identified, it turned out to be a protein". (Workers on actinomycin beware!) This no doubt explains why there is no treatment of molecular interactions between drugs and nucleic acids, which to my biased view seems rather a pity.

In part I the first three chapters, on model membranes, sugar transport receptors and ultrastructural studies, provide a useful and balanced introduction to the next four which are largely concerned with receptor identification and isolation, the prospects for which are succinctly stated in the quotation from Professor Burgen at the head of chapter 4. Here is a mine of valuable information about progress in several important areas which will commend itself to many a graduate student embarking on research. There follow three chapters concerned with conformational changes induced in proteins by drugs, the molecular pharmacology of acetylcholinesterase, and enzyme kinetics in the service of studies on structure-function relationships.

The scope of Part 2 is broader, with more concentration on the application of physical methods and mathematical

approaches to pharmacological problems. It is noteworthy that several authors of the more theoretically-based chapters are at pains to put their contributions into historical perspective and to emphasise that their calculations are intended to complement, but in no way replace, experimental investigations. There are chapters on nuclear magnetic resonance, electron spin resonance, optical activity, molecular orbital theory, and thermodynamics as applied to the study of drug-protein interactions. Another three chapters deal with anaesthetics, including the noble gases, primarily from the standpoint of discussing intermolecular forces involving simple drug molecules. They are reinforced by another chapter outlining the use of genetic variants to help probe the sites of action of inert anaesthetic gases and the binding of oxygen to haemoglobin. The two remaining chapters concern the induction of specific proteins by steroid hormones and the relative usefulness of lipids and proteins as models for studies in molecular pharmacology.

Notwithstanding delays caused by the failure of one author to deliver the goods (and he is accorded most commendably charitable treatment by the editor in his preface) there is evidence that a real effort was made to publish these books as promptly as possible. The bibliographies are sufficiently full to give the reader ready access to much if not most of the primary literature, but the rudimentary nature of the subject index places severe limitations on the value of the work as a reference source or guide. There is a much larger author index, but this is of limited value except as a means of verifying that a particular author's work has been cited. The intention is, however, clear enough that this publication is conceived as a collection of self-contained essays and in that light it will serve a useful purpose. Its appearance in two parts is in keeping

with the idea that each part may stand alone, but since no index is provided in part 1, and pages in part 2 are numbered to follow consecutively those of part 1, the two books are not so much companion volumes as moieties of a single work. The price seems rather high, particularly for part 1, but no doubt remains within the budget of libraries and our more affluent colleagues.

MICHAEL WARING

Industrial research

Co-operative Research in Industry: An Economic Study. By P. S. Johnson. Pp. vii+232. (Robertson: London, April 1973.) £4.

THE cooperative research associations have been a relatively neglected sector of the British R and D system. From their inception they have had to struggle hard for their existence and 14 have failed to survive. Their financial basis has always been somewhat precarious, as governments have usually striven to reduce their own contribution and persuade industry to cough up more. Industry, on the other hand, has been generally reluctant to increase its share, partly because of competitive considerations which militate in favour of captive in-house R and D, and partly because of backward attitudes. Only during and shortly after the two world wars did a more generous and far-sighted approach prevail. The RAs indeed owed their very existence to the first world war, which demonstrated forcefully the extent to which some branches of British industrial technology had fallen behind German competition.

The RAs have also been relatively neglected by economists. Not since Edwards's book in 1950 has there been any serious scholarly discussion of the fundamental economic problems of co-

operative industrial research in a capitalist competitive economy. Johnson's modest but highly competent book is therefore extremely welcome. It is especially valuable because it sets the problem firmly in a historical context, permitting one to assess the oscillations in government policy in a long-term perspective. In addition to the two introductory historical chapters, the book includes a valuable statistical survey of RA expenditure in each branch of industry and of the detailed breakdown of RA activities, with many useful tables. The chapters on economic analysis are complemented by the results of a field survey on the views of company research directors, particularly in relation to the growth of contract research. Finally, there is a very brief chapter on RAs in Europe and the United States. This is by far the weakest chapter in the book, which is a pity, as international comparisons would be particularly valuable in view of the growth of EEC-based industrial research.

In his conclusions on methods of financing RAs, Johnson generally welcomes the recent trends which he has demonstrated in his analysis, particularly the growth of contract finance, the switch from general government grants to specific government grants, and the trend towards payment for particular services, such as technical information, testing and so forth. Though his analysis is well argued and generally sound, and also reflects the views of many RA members, my own view is that he may perhaps be missing the wood for the trees to some extent. His survey shows fairly conclusively, as others have also shown, that most RAs are providing some very useful infrastructural technical services, particularly technical information, abstracting and translation services, standards, testing and safety work. They also do some very useful background research which benefits these activities and the research programmes of member firms. In these circumstances, though the extension of contract research is certainly welcome, it does seem to me that cheese-pairing on the small amount of government support for the RAs can be carried too far.

My main criticism of what is otherwise an admirable book is that Johnson does not relate his study of the RAs sufficiently to the general context of government policy for British industrial technology. The failure on the part of some governments to recognise their inescapable responsibility for the health of British science and technology, the efforts to dodge this responsibility in 1970-1972, the lack of a consistent policy for government laboratories and other aspects of the industrial science-technology system are an essential

Wanted: prolific big name

Nature receives many more books than it is able to review, but book publishers accept this and do not seem to withhold books which will not necessarily get reviewed. In certain cases of very expensive books or very small publishing companies, the publisher will make an informal enquiry about whether the book will be likely to be reviewed, since the expense of sending a copy for it not to be reviewed may be less easily bearable.

Recently, however, we have received a press release from a very large publisher on two books on the history of astronomy due later this

year. The aim is a "luxurious quality" of production and, quaintly, they can only be offered as a limited edition because of, among other reasons, "the wealth of illustrative material in the text and the nature of the subject".

The two books are £15 for 400 pages and £30 for 800 pages—not an outrageous price for well illustrated books, but "you will appreciate that the number of review copies . . . is very restricted. It will help us to judge between conflicting claims if you can specify the reviewer's name and the space you are prepared to devote".

background to the somewhat miserly approach to the RAs. Consequently, Johnson never asks how it was possible for successive governments to squander over £1,000 million on Concorde, while they were making great efforts to save a few hundred thousand pounds on the RAs. But this is the question which must be asked if we are to get value for money from government expenditure on R and D, if British industry is to be able to compete in the 1980s, and above all if British science and technology are to contribute substantially to the improvement of the quality of life for most people in these islands.

C. FREEMAN

Superconductive magic

Superconductive Tunnelling and Applications. By. L. Solymar. Pp. xii+406. (Chapman and Hall: London, June 1972.) £8.00.

THE story has all the ingredients of scientific legend: The phenomenon of superconductivity was discovered in 1911, but nearly half a century of effort by many of the leading lights of theoretical and experimental physics was required before it was well and truly understood. A necessary but not sufficient condition was the invention of quantum mechanics; an almost immediate consequence of that invention was the uncovering and elucidation, as early as 1928, of the phenomenon of tunneling. Not until the early sixties, after the development of a successful microscopic theory of superconductivity by Bardeen, Cooper, and Schrieffer, were these two phenomena brought together. And, lo, there materialised a veritable cornucopia of scientific fruit, with implications and applications ranging from purest physics to manifestly relevant technology. Moreover, the principal architects of this fertile union, Ivar Giaever and Brian Josephson, were mere students, not yet ordained in the doctoral priesthood of science. We have recently seen their achievement crowned with the Nobel Prize, hard on the heels of the same laurel for Bardeen, Cooper and Schrieffer.

If this view of the birth of tunneling in superconductors and of the Josephson effects seems a bit romantic, it is because I, after a decade of rummaging among the contents of the cornucopia, still find the whole affair rather magical. The task which Dr Solymar has set himself in writing this book is to present the results of the first decade or so of research on superconductive tunneling, magic included, in a form accessible to final year undergraduates and postgraduates in physics and electronic engineering, and to specialists in other fields who wish to learn what all the fuss has been about and perhaps to contribute to

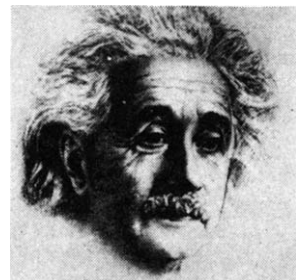
the continuing development of this field. This is no easy task. Much of the fundamental theoretical foundation is highly sophisticated. Although, after a decade of intensive work, it can fairly be argued that there remain few if any major mysteries in the field, contributions to knowledge of the basic phenomena continue to appear, and reports of advances in technologically relevant areas, especially of the Josephson effects, are proliferating beyond the ability of mortal man to keep track of, much less read and understand. Solymar's solution to the problem is to exploit a feature which superconductive tunneling shares with certain other physical systems, like the hydrogen atom: although one's study of the system can be carried to almost any level of complexity and sophistication, there exist levels at which real understanding, together with an appreciation for the beauty and the magic, can be conveyed to almost any student with a modicum of background.

He avoids the details of the microscopic theory, concentrates on phenomenological descriptions and models, and succeeds in presenting a comprehensive and understandable picture of the whole field in less than four hundred pages. It is all there: normal electron tunneling, Josephson tunneling, special effects, diagnostic and device applications, device fabrication, fluctuation phenomena, even a fascinating statistical note on the development of the field as measured by publications—number, journal distribution, country of national origin, and so on. It is a kind of Michelin green guide to the field. And for the reader who wants more than a two-paragraph or two-page introduction to the history of a particular château, there is a complete reference list (nearly 10^3 entries) to which he can go to learn everything that's known (or conjectured) on the subject.

As I understand it to be a reviewer's duty to carp, I am compelled to say that the relative emphasis on topics is sometimes not to my taste, that the urge to comprehensiveness has sometimes led the author to rather tedious listings of "X did Y experiment and measured Z", and that the proof reading leaves something to be desired (for example R. A. Ferrell's name consistently misspelled, the tin gap frequency reported at half its actual value (page 119), Figs 11.3 and 11.4 interchanged). On the whole, however, I think this book provides a remarkably good introduction to the field, one which I would (and will) recommend to many of my students or colleagues who wish to understand what superconductive tunneling is all about. I wish I had written it.

D. N. LANGENBERG

Einstein



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